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(54) Title: ALTERNATIVELY TARGETED ADENOVIRUS

(57) Abstract

The present invention provides a trimer comprising three monomers, each having an amino terminus of an adenoviral fiber protein and each having a trimerization domain. The trimer exhibits reduced affinity for a native substrate than a native adenoviral fiber trimer. The present invention further provides an adenovirus incorporating the trimer of the present invention. The present invention also provides a cell line expressing a non-native cell-surface receptor to which an adenovirus having a ligand for the receptor binds, and a method of propagating an adenovirus using the cell line. The present invention also provides a method of purifying an adenovirus having a ligand for a substrate from a composition comprising the adenovirus. The method involves exposing the composition to the substrate under conditions to promote the ligand to selectively bind the substrate. Subsequently, the composition not bound to the substrate is separated from the substrate, after which the bound adenovirus is eluted from the substrate. The present invention further provides a method of inactivating an adenovirus having a ligand recognizing a blood- or lymph-borne substrate by exposing the virus to the substrate. Within the blood or lymph, the ligand binds its substrate, thereby adsorbing the free virus from the blood or lymph. Additionally, the present invention provides a chimeric blocking protein comprising a substrate for an adenovirus fiber, and a method of interfering with adenoviral receptor binding by incubating an adenovirus with such chimeric blocking protein in a solution such that the chimeric blocking protein binds the fiber.

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ALTERNATIVELY TARGETED ADENOVIRUS

TECHNICAL FIELD OF THE INVENTION

The present invention relates to an alternately targeted adenovirus and includes methods for producing and purifying such viruses as well as protein modifications mediating alternate targeting.

BACKGROUND OF THE INVENTION

Adenoviral infection begins with the attachment of the virion to the target cell. The adenovirus attaches to two cellular surface proteins, both of which must be present for the virus to infect the target cell (Wickham et al., *Cell*, *73*, 309-19 (1993)). Wild-type adenovirus first binds the cell surface by means of a cellular adenoviral receptor (AR). One such AR is the recently-identified coxsackievirus and adenovirus receptor (CAR) (Bergelson et al., *Science*, *275*, 1320-23 (1997); Tanako et al., *Proc. Nat. Acad. Sci., U.S.A.*, *94*, 3352-56 (1997)); the MHC class I receptor also is an AR (Hong et al., *EMBO J.*, *16*(9), 2294-06 (1997)). After attachment to an AR, the virus attaches to a_V integrins, a family of a heterodimeric cell-surface receptors mediating interaction with the extracellular matrix and playing important roles in cell signaling (Hynes, *Cell*, *69*, 11-25 (1992)).

Following attachment to the cell surface, infection proceeds by receptor-mediated internalization of the virus into endocytotic vesicles (Svensson et al., *J. Virol.*, *51*, 687-94 (1984); Chardonnet et al., *Virology*, *40*, 462-77 (1970)). Within the cell, virions are disassembled (Greber et al., *Cell*, *75*, 477-86 (1993)), the endosome disrupted (Fitzgerald et al., *Cell*, *32*, 607-17 (1983)), and the viral particles transported to the nucleus via the nuclear pore complex (Dales et al., *Virology*, *56*, 465-83 (1973)).

The adenoviral virion is a non-enveloped icosahedron about 65-80 nm in diameter (Horne et al., *J. Mol. Biol.*, *1*, 84-86 (1959)). The adenoviral capsid comprises 252 capsomeres -- 240 hexons and 12 pentons (Ginsberg et al., *Virology*, *28*, 782-83 (1966)). The hexons and pentons are derived from three viral proteins (Maizel et al., *Virology*, *36*, 115-25 (1968); Weber et al., *Virology*, *76*, 709-24 (1977)). The hexon comprises three identical proteins of 967 amino acids each, namely polypeptide II (Roberts et al., *Science*, *232*, 1148-51 (1986)). The penton contains a base, which is bound to the capsid, and a fiber, which is non-covalently bound to and projects from, the penton base. Proteins IX, VI, and IIIa also are present in the adenoviral coat and are thought to stabilize the viral capsid (Stewart et al., *Cell*, *67*, 145-54 (1991); Stewart et al., *EMBO J.*, *12(7)*, 2589-99 (1993)).

The penton base is highly conserved among serotypes of adenovirus and (except for the enteric adenovirus Ad40) has five RGD tripeptide motifs (Neumann et al., *Gene*, *69*, 153-57 (1988)). In adenovirus, the RGD tripeptides apparently mediate adenoviral

binding to a_v integrins and endocytosis of the virion (Wickham et al. (1993), *supra*; Bai et al., *J. Virol.*, *67*, 5198-3205 (1993)).

The adenoviral fiber is a homotrimer of the adenoviral polypeptide IV (Devaux et al., *J. Molec. Biol.*, *215*, 567-88 (1990)). Structurally, the fiber has three discrete domains. The amino-terminal tail domain attaches non-covalently to the penton base. A relatively long shaft domain comprising a variable number of repeating 15 amino acid residues forming β-sheets extends outward from the vertices of the viral particle (Yeh et al., *Virus Res.*, *33*, 179-98 (1991)). Lastly, roughly 200 amino-acid residues at the carboxy-terminal form the knob domain. Functionally, the knob mediates primary viral binding to the cellular AR and fiber trimerization (Henry et al., *J. Virol.*, *68(8)*, 5239-46 (1994)). Hence, the trimerization domain of a fiber is a ligand for a cell-surface receptor native for the adenoviral serotype. The trimerization domain also appears necessary for the tail of the fiber to properly associate with the penton base (Novelli et al., *Virology*, *185*, 365-76 (1991)). In addition to recognizing cell ARs and binding the penton base, the fiber protein contributes to serotype integrity and mediates nuclear localization.

Fiber proteins from different adenoviral serotypes differ considerably. For example, the number of 15 amino-acid β-sheet repeats differs between adenoviral serotypes (Green et al., *EMBO J.*, *2*, 1357-65 (1983)). Moreover, the knob regions from the closely related Ad2 and Ad5 serotypes are only 63% similar at the amino acid level (Chroboczek et al., *Virology*, *186*, 280-85 (1992)), and Ad2 and Ad3 fiber knobs are only 20% identical (Signas et al., *J. Virol.*, *53*, 672-78 (1985)). In contrast, the penton base sequences are 99% identical. Despite these differences in the knob region, a sequence comparison of even the Ad2 and Ad3 fiber genes demonstrates distinct regions of conservation, most of which are also conserved among the other human adenoviral fiber genes.

A number of factors present the adenovirus as an attractive vector choice for use in a variety of gene transfer applications (e.g., cellular protein production, therapy, academic study, etc.). For example, the adenovirus is a superior expression vector. Recombinant adenovirus can be produced in high titers (e.g., about 10^{13} viral particles/ml), and adenoviral vectors can transfer genetic material to non-replicating, as well as replicating, cells (in contrast with retroviral vectors). The adenoviral genome can be manipulated to carry a large amount of exogenous DNA (up to about 7.5 kb), and the adenoviral capsid can potentiate the transfer of even longer sequences (Curiel et al., *Hum. Gene Ther.*, *3*, 147-54 (1992)). Additionally, several features suggest that adenoviruses represent a safe choice for gene transfer, a particular concern for therapeutic applications. For example, adenoviruses do not integrate into the host cell chromosome, thus minimizing the likelihood that an adenoviral vector will interfere with normal cell function. Moreover, adenoviral infection does not correlate with human malignancy, and recombination of the

adenoviral genome is rare. Due to these advantages, clinicians have employed adenoviral vectors safely as a human vaccine and for gene therapy for many years.

Based on the popularity of adenoviral vectors, efforts have been made to increase the ability of adenovirus to enter certain cells, e.g., those few cells it does not infect, an approach referred to as "targeting" (see, e.g., International Patent Application WO 95/26412 (Curiel et al.), International Patent Application WO 94/10323 (Spooner et al.), U.S. Patent 5,543,328 (McClelland et al.), International Patent Application WO 94/24299 (Cotten et al.)). Of course, while the ability to target adenoviruses to certain cell types is an important goal, far more desirable is an adenovirus which infects only a desired cell type, an approach referred to as "exclusive targeting." However, to exclusively target a virus, its native affinity for host cell ARs must first be abrogated, producing a recombinant adenovirus incapable of productively infecting the full set of natural adenoviral target cells. Efforts aimed at abrogating native adenoviral cell affinity have focused logically on changing the fiber knob. These efforts have proven disappointing, largely because they fail to preserve the important fiber protein functions of stable trimerization and penton base binding (Spooner et al., *supra*). Moreover, replacement of the fiber knob with a cell-surface ligand (McClelland et al., *supra*) produces a virus only suitable for infecting a cell type having that ligand. Such a strategy produces a virus having many of the same targeting problems associated with wild-type adenoviruses (in which fiber trimerization and cellular tropism are mediated by the same protein domain), thus decreasing the flexibility of the vector. Moreover, due to the necessity of having a host cell, and the integral connection between the fiber trimization and targeting functions, obtaining a mutant virus with substituted targeting is difficult. For example, removing the fiber knob and replacing it with a non-trimerizing ligand (e.g., McClelland et al., *supra*) results in a virus lacking appreciable fiber protein. As such, there is currently a need for an adenoviral fiber having reduced affinity for natural ARs but retaining fiber trimerization and penton base-binding function.

While exclusive adenoviral targeting requires reducing native cellular tropism, the abrogation of natural targeting also reduces the ability of the virus to infect cell lines normally employed for its propagation (e.g., 293 cells) (see Curiel et al., *supra*). One published attempt at surmounting this barrier fortuitously employed a cell line expressing the relevant cell surface binding site (McClelland et al., *supra*), and thus did not address this central concern. However, many cell lines do not express important cellular receptors. Moreover, many available cell lines expressing potentially useful cell surface binding sites are inadequate for production of recombinant adenoviruses, especially viruses useful for clinical application (e.g., cell lines harboring and expressing the essential adenoviral immediate early genes from the E1, E2, and/or E4 regions of the genome). There is thus a need for a cell line, and a means of producing a cell line, which

can propagate and package a recombinant adenovirus substantially incapable of productively infecting cells via native ARs.

Typical protocols for purifying viral vectors from packaging cell lysates involve centrifuging the viruses through a $CsCl_2$ gradient one or more times. While such methods adequately isolate viruses, they generally require considerable material ($CsCl_2$) and are therefore relatively inefficient. Moreover, such protocols are not readily amenable to high throughput application, presenting a significant barrier to economic development of viral vectors on a commercial scale. Other methods involving column purification do not bind the viruses specifically (Shabram et al., *Hum. Gene Ther.*, *8*, 453 (1997); Huyghe et al., *Hum. Gene Ther.*, *6*, 1403 (1995)), often resulting in an unacceptable amount of contaminants compared to the purity obtainable in affinity purification of other materials. Thus, there is a need for an efficient method of purifying and isolating recombinant viral vectors.

In many applications involving *in vivo* delivery of viral vectors, it is desirable to contain infection (and gene delivery) to the tissue of interest. For example, the threat of systemic infection and delivery of a biologically active gene represents a significant concern to gene therapy applications. Moreover, ectopic expression of a transgene would spoil many experimental applications. While, in theory, host blood cells can express proteins mediating the clearing of foreign substances, such as adenoviruses (News and Comment, *Science*, *275*, 744-45 (1997)), engineering such cells and producing them in the host are difficult and intrusive. Moreover, while antibodies directed against the adenoviral hexon can inactivate the virus (Toogood et al., *J. Gen. Virol.*, *73*, 1429-35 (1992)), efficient protocols for delivering a sufficient quantity of anti-hexon antisera to the gene transfer recipient in time to reduce or prevent ectopic viral infection have not been forthcoming, and such a strategy can actually interfere with gene transfer protocols by blocking infection in desired tissues. Thus, there is a need for a method of inactivating recombinant viral vectors leaving the desired locus of delivery within a host animal.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a trimer comprising three monomers, each having an amino terminus of an adenoviral fiber protein and each having a trimerization domain. The trimer exhibits reduced affinity for a native substrate than a native adenoviral fiber trimer. The present invention further provides an adenovirus incorporating the trimer of the present invention. The present invention also provides a cell line expressing a non-native cell-surface receptor to which an adenovirus having a ligand for the receptor binds, and a method of propagating an adenovirus using the cell line.

The present invention also provides a method of purifying an adenovirus having a ligand for a substrate from a composition comprising the adenovirus. The method

to selectively bind the substrate. Subsequently, the composition not bound to the substrate is separated from the substrate, after which the bound adenovirus is eluted from the substrate.

The present invention further provides a method of inactivating an adenovirus having a ligand recognizing a blood- or lymph-borne substrate by exposing the virus to the substrate. Within the blood or lymph, the ligand binds its substrate, thereby adsorbing the free virus from the blood or lymph.

Additionally, the present invention provides a chimeric blocking protein comprising a substrate for an adenovirus fiber, and a method of interfering with adenoviral receptor binding by incubating an adenovirus with such chimeric blocking protein in a solution such that the chimeric blocking protein binds the fiber.

The present invention is useful in a variety of applications, *in vitro* and *in* vivo, such as therapy, for example, as a vector for delivering a therapeutic gene to a cell with minimal ectopic infection. Specifically, the present invention permits more efficient production and construction of safer vectors for gene therapy applications. The present invention is also useful as a research tool by providing methods and reagents for the study of adenoviral attachment and infection of cells and in a method of assaying receptor-ligand interaction. Similarly, the recombinant fiber protein trimers can be used in receptor-ligand assays and as adhesion proteins *in vitro* or *in vivo*. Additionally, the present invention provides reagents and methods permitting biologists to investigate the cell biology of viral growth and infection. Thus, the vectors of the present invention are highly useful in biological research.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B depict the three-dimensional structure of an adenoviral knob protein (serotype 5). Figure 1A is a ribbon diagram representing β-sheets and the loops interconnecting the sheets. Figure 1B is a filled-in diagram taking into account the relative sizes of the amino acid residues.

Figure 2 is a sequence comparison between adenoviral serotypes.

Figures 3A-3C depict vectors for creating recombinant adenoviral fiber trimers having non-native trimerization domains. Figure 3A depicts pAcT5S7GCNTS.PS.LS.X. Figure 3B depicts pAcT5sigDel.TS.PS.LS. Figure 3C depicts pAcT5S7sigDel.TS.PS.LS.

Figure 4 depicts pAcT5sigDel.GFP.TS.PS.LS, a vector containing a gene encoding a fiber-sigDel-GFP chimera.

Figures 5A-5D depict vectors useful for the construction of recombinant adenovirus vectors containing fiber trimers having non-native trimerization domains. Figure 5A depicts pAS pGS HAAV. Figure 5B depicts pAS pGS pK7. Figure 5C depicts pAS T5S7sigDelpGS.HAAV. Figure 5D depicts pAST5S7sigDel.GFP.pGS.pK7.

Figures 6A-6D represent vectors used in the construction of fiber trimers having non-native trimerization domains. Figure 6A represents pAcPig4KN. Figure 6B represents pAcPigKN D363E. Figure 6C depicts pAcPigKN N437D. Figure 6D depicts pAcPig4KN(FLAG).

Figures 7A-7B represent vectors employed in creating a fiber trimer having a non-native trimerization domain. Figure 7A depicts PNS F5F2K. Figure 7B depicts pNS Pig4.SS.

Figures 8A-8C represent vectors useful for creating an adenoviral vector having a chimeric fiber trimer comprising a mutant NADC-1 knob lacking native receptor-binding ability and containing a functional non-native ligand. Figure 8A depicts pAcPig4KN D363E N437D. Figure 8B depicts pAcPig4KN D363E N437D HAAV. Figure 8C depicts pNS Pig4 D363E N437D HAAV SS.

Figures 9A-9B represent vectors useful for creating chimeric blocking proteins of the present invention able to interfere with native adenoviral receptor binding. Figure 9A depicts pACSG2-sCAR. Figure 9B depicts pACSG2-sCAR-HAAV.

Figures 10A-10B represent vectors useful for creating chimeric blocking proteins able to form trimers interfering with native adenoviral receptor binding. Figure 10A depicts pAcSG2sCAR.sigDel. Figure 10B depicts pAcSG2-sCARsigDel (HAAV).

Figures 11A-11E depict vectors useful for creating construction of adenovirus vectors having specific non-native ligands. Figure 11A depicts pBSSpGS. Figure 11B depicts pBSS pGS (RKKK)2. Figure 11C depicts pNSF5F2K(RKKK)2. Figure 11D depicts pBSSpGS (FLAG). Figure 11E depicts pNS F5F2K(FLAG).

Figures 12A-12E represent vectors useful for creating a cell line expressing a non-native cell surface binding site substrate. Figure 12A depicts pHOOK3. Figure 12B depicts pRC/CMVp-Puro. Figure 12C pScHAHK. Figure 12D depicts pNSE4GLP.

Figures 13A-13D represent vectors useful for creating a fiber-expressing cell line for the production of targeted adenovirus particles. Figure 13A depicts pCR2.1-TOPO+fiber. Figure 13B depicts pKSII Fiber. Figure 13C depicts pSMTZeo-DBP. Figure 13D depicts pSMTZeo-Fiber.

Figure 14 depicts pAdE1(Z)E3/E4(B), a plasmid useful for the construction of targeted adenovirus particles having genomes encoding chimeric fibers.

Figures 15A-15E illustrate the locations of mutations within adenoviral knobs which interfere with ligand binding. Figure 15A is a top view, Figure 15B a side view, and Figure 15C a bottom view of the knob illustrating the location of the 3D9 mutation. Figure 15D is a top view, Figure 15E a side view, and Figure 15F a bottom view of the knob illustrating the locations of the CD loop mutation, the FG loop mutation, and the IJ mutation.

Figure 16 depicts a vector useful for the construction of a recombinant adenovirus containing a short-shafted fiber and a mutant fiber knob exhibiting reduced affinity for its native receptor.

Figures 17A-17B depict vectors useful for constructing a cell line able to replicate adenoviruses lacking native cell-binding function (but targeted for a pseudo-receptor). Figure 17A depicts pCANTAB5E(HA). Figure 17B depicts pScFGHA.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

An **adenovirus** is any virus of the genera *Mastadenoviridae* or *Aviaadenoviridae*, and can be of any serotype within those genera. Adenoviral stocks that can be employed as a source of adenovirus or adenovirus coat protein such as penton base and/or fiber protein can be amplified from the adenovirus serotypes currently available from American Type Culture Collection (ATCC, Rockville, MD), or from any other source.

A **ligand** is any species selectively binding an identifiable substrate.

Native refers to a protein or property of an unmodified virus or cell. Thus, a non-native protein can be a modified or mutated protein differing from its native homologue within the virus or cell. Alternatively, a non-native protein can be a protein having no native homologue within the virus or cell.

An **AR** refers to an adenoviral receptor. In particular, an AR is a ligand binding the mastadenoviral knob.

A first species is **selectively bound** to a substrate if it binds the substrate with greater affinity than a second species. The first species is not selectively bound if binds the substrate with the same or lesser affinity than the second species, even if the first species binds with some affinity.

Trimers

The present invention provides a trimer comprising three monomers (e.g., at least a portion of each of three adenoviral fiber monomers), each having an amino terminus derived from an adenoviral fiber protein and each having a trimerization domain. The inventive trimer exhibits reduced affinity for a native substrate, such as an antibody, a cellular binding cite, etc. (i.e., native to the serotype from which the shaft, and particularly the amino-terminus, is drawn) as compared to a native adenoviral fiber trimer. The trimer can be a homotrimer or a heterotrimer of different fiber monomers. Any modification of the monomeric units reducing the affinity of the resulting trimer for its native cell surface binding site (i.e., a native AR) is within the scope of the invention. Preferably, the reduction in affinity is a substantial reduction in affinity (such as at least an order of magnitude, and preferably more) relative to the unmodified corresponding fiber.

As mentioned, where a trimerization domain is itself a ligand for a native cell surface binding site, trimers possessing such trimerization domains present some of the same problems for targeting as native adenoviral fiber trimerization domains. Therefore, the trimerization domain of a monomer incorporated into the trimer of the invention preferably is not a ligand for the CAR or MHC-1 cell surface domains, or antibodies recognizing the fiber. Most preferably, the non-native trimerization domain is not a ligand for any native mammalian cell-surface binding site, whether the site is an AR or other cell surface binding site. As is discussed herein, adenoviruses incorporating such trimers exhibit reduced ability to appreciably infect their native host cells, and can serve as efficient source vectors for engineering selectively targeted vectors. Therefore, while the trimerization domain preferably is not a ligand for a cell surface binding site, the entire trimer can be such a ligand (by virtue of a non-native ligand as discussed herein). Moreover, the trimerization domain can be a ligand for a substrate other than a native cell surface binding site, as such trimerization-ligands do not present the same concern for cell targeting as do trimerization domains which are ligands for cell surface binding sites. Thus, for example, the non-native trimerization domain can be a ligand for a substrate on an affinity column, on a blood-borne molecule, or even on a cell surface when it is not a native cell-surface binding site (e.g., on a cell engineered to express a substrate cell surface protein not native to the unmodified cell type).

A monomer for inclusion into a trimer can be all or a part of a native adenoviral fiber monomeric protein. For example, a modified monomer can lack a sizable number of residues, or even identifiable domains, as herein described. For example, a monomer can lack the native knob domain; it can lack one or more native shaft β-sheet repeats, or it can be otherwise truncated. Thus, a monomer can have any desired modification so long as it trimerizes. Furthermore, a monomer preferably is not modified appreciably at the amino terminus (e.g., the amino-terminus of a monomer preferably consists essentially of the native fiber amino-terminus) to ensure that the resultant trimer interacts properly with the penton base. Hence, the present invention also provides a composition of matter comprising a trimer of the present invention and an adenoviral penton base. Preferably, the trimer and the penton base associate much in the same manner as wild-type fibers and penton bases. Of course, while the trimer comprises modified fiber monomers, the penton base can also be modified, for example, to include a non-native ligand, for example as is described in U.S. Patent 5,559,099.

Mutant Knobs

A fiber monomer for incorporation into the trimer of the present invention has a trimerization domain which binds a native mammalian AR (i.e., an AR native for the adenoviral serotype of interest) with less affinity than a native adenoviral fiber. Trimers

incorporating such monomers preferably are not ligands for their native cellular binding sites. The monomers can be modified in any manner suitable for reducing the affinity of the fiber for native AR while permitting the monomers to trimerize. For example, in one embodiment, the trimerization domain is a modified adenoviral fiber knob domain lacking a native receptor-binding amino acid. Any native amino-acid residue mediating or assisting in the interaction between the knob and a native cellular AR is a suitable amino acid for mutation or deletion from the monomer. Moreover, the knob domain can lack any number of such native receptor-binding amino acids, so long as, in the aggregate, the monomers associate to form a trimer of the present invention.

Native amino acid residues for modification or deletion can be selected by any method. For example, the sequences from different adenoviral serotypes can be compared to deduce conserved residues likely to mediate AR-binding. Alternatively or in combination, the sequence can be mapped onto a three dimensional representation of the protein (such as the crystal structure) to deduce those residues most likely responsible for AR binding. These analyses can be aided by resorting to any common algorithm or program for deducing protein structural functional interaction. Alternatively, random mutations can be introduced into a cloned adenoviral fiber expression cassette. One method of introducing random mutations into a protein is via the Taq polymerase. For example, a clone encoding the fiber knob (see, e.g., SEQ ID NO:9; Roelvink et al., *J. Virol., 70*, 7614-21 (1996)) can serve as a template for PCR amplification of the adenoviral fiber knob, or a portion thereof. By varying the concentration of divalent cations in the PCR reaction, the error rate of the transcripts can be largely predetermined (see, e.g., Weiss et al., *J. Virol., 71*, 4385-94 (1997); Zhou et al., *Nucl. Acid. Res., 19*, 6052 (1991)). The PCR products then can be subcloned back into the template vector to replace the sequence within the fiber coding sequence employed as a source for the PCR reaction, thus generating a library of fibers, some of which will harbor mutations which diminish native AR binding while retaining the ability to trimerize.

A monomer lacking one or more amino acids, as herein described, can optionally comprise a non-native residue (e.g., several non-native amino acids) in addition to or in place of the missing native amino acid(s); of course, alternatively, the native amino acid(s) can simply be deleted from the knob. Preferably, the amino-acid is substituted with another non-native amino acid to preserve topology and, especially, trimerization. Moreover, if substituted, the replacement amino acid preferably confers novel qualities to the monomer. For example, to maximally ablate binding to the native AR, a native amino acid can be substituted with a residue (or a plurality of residues) having a different charge. Such a substitution maximally interferes with the electrostatic interaction between native adenoviral knob domains and cellular ARs. Similarly, a native amino acid can be substituted with a heavier residue (or a plurality of residues) where possible. Heavier

residues have longer side-chains; hence, such a substitution maximally interferes with the steric interaction between native adenoviral knob domains and cellular ARs.

Non-native Trimerization Domains

In another embodiment, the trimer includes modified monomers which are chimeric adenoviral fiber polypeptides. A suitable chimeric monomer lacks all or a portion of the trimerization domain native to the source adenoviral serotype. The trimerization domain of such a monomer can be deleted from the virus, or the trimerization domain can be ablated by inserting or substituting non-native amino acids into the domain. Of course, a monomer lacking the native trimerization domain can also lack the entire native knob. Because the native trimerization domain is a ligand for a native AR, a trimer of chimeric adenoviral fiber monomers lacking the native trimerization domain binds its native AR with less affinity than the native adenoviral fiber.

For the chimeric monomers to form a trimer of the present invention, they must incorporate a replacement (i.e., non-native) trimerization domain. To maximally promote the targeting of the virus, preferably the non-native trimerization domain is not a ligand for a mammalian cell-surface receptor, or any cell-surface receptor. Any domain able to form homotrimers is a suitable trimerization domain for inclusion into the trimers of the present invention, and several are known in the art. For example, a chimeric monomer can include the trimerization domain from the heat shock factor (HSF) protein of *K. lactis* (Sorger and Nelson, *Cell*, *59*, 807 (1989)), trout axonal dynein (Garber et al., *EMBO J.*, *8*, 1727 (1989)), parainfluenza virus hemagglutanin protein (Coelingh et al., *Virology*, *162*, 137 (1988)), the sigma 1 protein of reovirus type 1 (Strong et al., *Virology*, *184*, 12 (1991)), or other suitable trimer. Alternatively, a chimeric monomer can include a modified leucine-zipper motif. Leucine zippers comprise heptad repeats of leucines, which mediate dimerization. However, replacement of one or more leucine with isoleucine results in stable trimerization of the domains. An example of such a modified leucine zipper motif is the 32 amino acid GCN4p-II trimer (Harbury et al., *Science*, *262*, 1401 (1993)).

Of these trimerization domains, the reovirus sigma 1 trimerization domain is preferred. This protein contains 17 alpha helical heptad repeats, reminiscent of the coiled-coil trimer structure of the aforementioned mutant isoleucine zipper domains (Harbury et al., *Nature*, *371*, 80-83 (1994)). Fiber chimeras containing the sigma 1 domain can thus protrude farther from the virus than corresponding chimeras containing shorter trimerization domains. An advantage of the reovirus sigma 1 trimerization domain over a mutant leucine-zipper (e.g., GCN4) domain is that the sigma 1 domain is 22 nm long (Fraser et al., *J. Virol.*, *64*, 2990-3000 (1990)) whereas GCN4 domain is only 5 nm

long (Harbury et al., *supra*). An additional advantage to employing the reovirus sigma 1 attachment protein is that, unlike the adenoviral shaft protein, it exhibits intrinsic trimerization propensity (Leone et al., *Virology, 182*, 336-45 (1991)). As fiber length appears to increase the efficiency and specificity of adenoviral-cell attachment (Roelvink et al., *J. Virol., 70*, 7614-21 (1996)), longer fibers possible with the sigma 1 domain are preferred to other chimeric fibers.

A chimeric monomer can alternatively include a knob domain from another adenoviral serotype. For example, the trimerization domain can be replaced with a mutated knob from an adenoviral serotype capable of productive infection within the host species (e.g., a mutant knob of Ad3 containing a mutation in the HI loop). Alternatively, it can be replaced with a knob from a serotype not capable of productive infection within the host species. For example, the fiber knob of a mammalian adenoviral serotype can be replaced with a knob from an avian serotype. While the avian knob mediates trimerization of the fiber proteins, it is likely unable to recognize a mammalian AR; hence, such chimeric fibers lack the native ability to bind the native host AR. Similarly, the fiber knob of one mammalian adenoviral serotype can be replaced with a knob from another mammalian serotype. In this regard, a modified or unmodified knob from the porcine adenovirus NADC-1 fiber is a preferred domain, as the NADC-1 is well characterized. The NADC-1 knob has identifiable ligands, e.g., galectin (which binds galactose), and LDZ and RGD peptides, (which bind integrins) (see, e.g., Hirabayashi et al., *J. Biol. Chem., 266*, 13648-53 (1991)). Thus, chimeric human adenoviral fibers having NADC-1 knobs with such mutations can form trimers and associate with the penton base, but they bind native cell-surface receptors with reduced affinity.

The non-native trimerization domain can be ligated to the native fiber monomer at any suitable site, so long as the monomers can trimerize properly (i.e., be capable of interacting with an adenoviral penton base). For example, the domain can be inserted into the native knob to disrupt knob topology. Alternatively, the trimerization domain can be inserted after any of the 15 amino acid shaft repeats, preferably after the 7^{th}, 15^{th}, or 22^{d} repeats to mimic native adenoviral shaft size. Where the non-native trimerization domain is inserted into the adenoviral shaft, it can form the carboxy-terminus of the chimeric protein, or it can be inserted into the middle of the amino acid sequence. Moreover, any number of trimerization domains can be so inserted into the fiber monomer, so long as the resulting trimer properly associates with the penton base.

Blocking Domain

Another suitable chimeric monomer has a novel domain blocking the ligand for the native host AR. The blocking domain is any peptide which can be tightly bound to the native ligand. (See, e.g., Hong et al., *EMBO J., 16*, 2294-2306 (1997)). In other

words, the blocking domain is a substrate to which the (native or modified) fiber monomer ligand selectively binds. Desirably, the ligand-substrate interaction occurs at least immediately upon viral production and effectively continues until the fiber trimer is destroyed. Because the native ligand binds the blocking domain, the ligand is incapable of binding its native substrate on cell surfaces. Because the native trimerization domain is a ligand for a native AR, trimers of chimeric adenoviral fiber monomers having such blocking domains bind the native AR with less affinity than a native adenoviral fiber.

The blocking domain can be at any position on the adenovirus to bind the native ligand without appreciably affecting trimerization or penton base interaction. For example, the blocking domain can be appended to the above-referenced β-sheets or loops, either by fusion within the reading frame, by covalent post-translational modification, etc. Alternatively, the blocking domain can be appended to another portion of the monomer, such as the shaft. The blocking domain can also include a linker or spacer polypeptide to afford an opportunity for the blocking domain to interact with the native ligand. If the blocking domain is attached via such a spacer, the spacer can include a protease recognition site for subsequent cleavage, as described herein.

Preparation

The monomers for inclusion into the trimers of the present invention can be produced by any suitable method. For example, the mutant fiber protein can be synthesized using standard direct peptide synthesizing techniques (e.g., as summarized in Bodanszky, *Principles of Peptide Synthesis* (Springer-Verlag, Heidelberg: 1984)), such as via solid-phase synthesis (see, e.g., Merrifield, *J. Am. Chem. Soc.*, *85*, 2149-54 (1963); Barany et al., *Int. J. Peptide Protein Res.*, *30*, 705-739 (1987); and U.S. Patent 5,424,398). Alternatively, site-specific mutations (such as replacing the knob with a non-native trimerization domain, removing, replacing, or mutating the AR-binding residues, or adding a blocking domain, as herein described) can be introduced into the monomer by ligating into an expression vector a synthesized oligonucleotide comprising the modified site. Alternatively, a plasmid, oligonucleotide, or other vector encoding the desired mutation can be recombined with the adenoviral genome or with an expression vector encoding the monomer to introduce the desired mutation. Oligonucleotide-directed site-specific mutagenesis procedures also are appropriate(e.g., Walder et al., *Gene*, *42*, 133 (1986); Bauer et al., *Gene*, *37*, 73 (1985); Craik, *Biotechniques*, 12-19 (1995); U.S. Patents 4,518,584 and 4,737,462). However engineered, the DNA fragment encoding the modified monomer can be subcloned into an appropriate vector using well known molecular genetic techniques. The fragment is then transcribed and the peptide subsequently translated *in vitro* within a host cell. Any appropriate expression vector (e.g., Pouwels et al., *Cloning Vectors: A Laboratory Manual* (Elsevior, NY: 1985)) and

corresponding suitable host cells can be employed for production of recombinant peptides. Expression hosts include, but are not limited to, bacterial species, mammalian or insect host cell systems including baculovirus systems (e.g., Luckow et al., *Bio/Technology*, *6*, 47 (1988)), and established cell lines such 293, COS-7, C127, 3T3, CHO, HeLa, BHK, etc. An especially preferred expression system for preparing modified fibers of the invention is a baculovirus expression system (Wickham et al., *J. Virol.*, *70*, 6831-38 (1995)) as it allows the production of high levels of recombinant proteins. Of course, the choice of expression host has ramifications for the type of peptide produced, primarily due to post-translational modification.

Once produced, the monomers are assayed for fiber protein activity. Specifically, the ability of the monomers to form trimers, interact with the penton base, and interact with native ARs is assayed. Any suitable assay can be employed to measure these parameters. For example, as improperly folded monomers are generally insoluble (Scopes, "Protein Purification" (3d Ed., 1994), Chapter 9, p. 270-82 (Springer-Verlag, New York)), one assay for trimerization is whether the recombinant fiber is soluble. Determining solubility of the fiber is aided if an amount of radioactive amino-acid is incorporated into the protein during synthesis. Lysate from the host cell expressing the recombinant fiber protein can be centrifuged, and the supernatant and pellet can be assayed via a scintillation counter or by Western analysis. Subsequently, the proteins within the pellet and the supernatant are separated (e.g., on an SDS-PAGE gel) to isolate the fiber protein for further assay. Comparison of the amount of radioactivity in the fiber protein isolated from the pellet vis-à-vis the fiber protein isolated from the supernatant indicates whether the mutant protein is soluble. Alternatively, trimerization can be assayed by using a monoclonal antibody recognizing only the amino portion of the trimeric form of the fiber (e.g., via immunoprecipitation, Western blotting, etc.). One such antibody is described in International Patent Application WO 95/26412, and others are known in the art. A third measure of trimerization is the ability of the recombinant fiber to form a complex with the penton base (Novelli and Boulanger, *Virology*, *185*, 1189 (1995)), as only fiber trimers can so interact. This propensity can be assayed by co-immunoprecipitation, gel mobility-shift assays, SDS-PAGE (boiled samples run as monomers, otherwise, they run as larger proteins), etc. A fourth measure of trimerization is to detect the difference in molecular weight of a trimer as opposed to a monomer. For example, a boiled and denatured trimer will run as a lower molecular weight than a non-denatured stable trimer (Hong and Angler, *J. Virol.*, *70*, 7071-78 (1996)).

A trimeric recombinant fiber must also be assayed for its ability to bind native ARs. Any suitable assay that can detect this is sufficient for use in the present invention. A preferred assay involves exposing cells expressing a native AR (e.g., 293 cells) to the recombinant fiber trimers under standard conditions of infection. Subsequently, the cells

are exposed to native adenoviruses, and the ability of the viruses to bind the cells is monitored. Monitoring can be by autoradiography (e.g., employing radioactive viruses), immunocytochemistry, or by measuring the level of infection or gene delivery (e.g., using a reporter gene). In contrast with native trimers which reduce or substantially eliminate subsequent viral binding to the 293 cells, those trimers not substantially reducing the ability of native adenoviruses to subsequently bind the cells are trimers of the present invention. The reduction of interference with subsequent viral binding indicates that the trimer is itself not a ligand for its native mammalian AR, or at least binds with reduced affinity.

Alternatively, a vector including a sequence encoding a mutated fiber (or a library of putative mutated fibers, such as described herein) can be introduced into a suitable host cell strain to express the fiber protein. For high-efficiency screening, preferably the host cells are bacteria. Where bacteria are employed as host cells, mutants can be identified by assaying the ability to bind the soluble CAR protein. For example, a replica of the bacterial plate (e.g., on a nitrocellulose filter lift) can be cultured in a suitable medium to induce expression from the vector. Subsequently, the filter is exposed to a solution suitable for lysing the bacteria adhering to it, and the probed with a radiolabled CAR protein. Preferably, the filter is first "blocked" with a high protein solution to minimize nonspecific adherence of the CAR probe to the filter. After the hybridization, the filter is exposed to film to identify colonies expressing fiber proteins that bind the CAR. Those colonies not hybridizing to the radiolabeled CAR probe (or binding with reduced affinity as indicated by weaker signal) potentially express fiber monomers of the present invention. Because a reduction in CAR-binding could be due to either selective ablation of the ligand or structural modification affecting trimerization, mutant fibers identified as non-CAR binding by such a bacterial library screen must be assayed for the ability to trimerize, as described above.

Blocking Proteins

As an alternate means for reducing native viral tropism, the present invention provides a chimeric blocking protein comprising a substrate for an adenovirus fiber. The chimeric blocking protein can include any suitable domain having a substrate recognized by the ligand on the adenoviral fiber. For example, for interfering with the receptor-binding of a wild-type adenovirus, the chimeric blocking protein can comprise the extracellular domain of the CAR cell-surface protein (Bergelson et al., *Science, 275*, 1320-23 (1997); Tomko et al., *Proc. Nat. Acad. Sci. U.S.A., 94*, 3352-56 (1997)), the extracellular domain for the MHC class I receptor (Hong et al., *EMBO J., 16*(9), 2294-06 (1997)), or other similar extracellular substrate domain for an AR. Moreover, for interfering with the substrate-binding of recombinant adenoviruses, such as adenoviruses

having chimeric fiber trimers as described herein, the blocking protein can comprise a substrate recognized by a ligand present on the trimer. While, as mentioned, the chimeric blocking protein can comprise domains from cell-surface proteins, typically it is not itself a cell-surface protein. Instead, the chimeric blocking protein is preferably a free soluble protein able to interact with an adenovirus in solution.

A chimeric blocking protein of the present invention affords a method of interfering with adenoviral receptor-binding by incubating an adenovirus with the chimeric blocking protein in a solution such that the chimeric blocking protein binds the ligand present on the adenoviral fiber. The virus and the chimeric blocking protein can be incubated for any length of time, and under any suitable conditions, to promote the ligand on the fiber to bind the substrate on the chimeric blocking protein. The parameters of time, temperature, and solution chemistry suitable for promoting selective binding between the fiber ligand and the chimeric blocking protein substrate can vary according to the affinity with which the ligand selectively binds the substrate. Generally, where known ligand-substrate systems are employed, these parameters are also known. Where novel ligand-substrate systems are employed, however, the binding conditions can, in large measure, be predetermined as discussed herein (e.g., by employing such conditions when screening the protein library for the novel ligand-substrate interaction). However, preferably the concentration of the chimeric blocking proteins is sufficient to saturate the cell-surface ligands present on the fibers of the adenovirus during the incubation.

In addition to including a domain having a substrate recognized by the ligand on an adenoviral fiber, a chimeric blocking protein also can have other domains. For example, the protein can include domains to promote secretion (see, e.g., Suter et al., *EMBO, J., 10*, 2395-2400 (1991); Beutler et al., *J. Neurochem., 64*, 475-81 (1995)), thus aiding in the collection of free chimeric blocking proteins from cells producing the protein. Additionally, the chimeric blocking protein preferably further includes a ligand domain (i.e., a ligand in addition to the substrate for the viral knob), such as those ligands described herein. The presence of a ligand on the chimeric blocking protein, notably peptide tags and other similar sequences, facilitates purification and identification of the chimeric blocking protein after production. A more preferred ligand is one recognizing a cell surface binding site or other substrate, as discussed herein. Such blocking proteins function as "bi-specific" molecules for altering adenoviral receptor binding. For example, where a chimeric blocking protein includes a ligand for a cell-surface binding site, the blocking protein is able to effect selective targeting of the adenovirus by interfering with fiber-mediated receptor binding while directing novel targeting through the ligand present on the chimeric blocking protein. Thus, the present invention provides a method of directing adenoviral targeting by incubating an adenovirus with a chimeric blocking protein having a ligand recognizing a substrate present on a cell surface binding site in a

solution such that the chimeric blocking protein binds the adenoviral fiber to form a complex, and thereafter exposing the complex to a cell having a substrate for the ligand.

In addition to including a domain having a substrate recognized by the ligand on an adenoviral fiber (and possibly a non-adenoviral ligand domain), the chimeric blocking protein also can include a trimerization domain, such as those trimerization domains discussed herein. The presence of such trimerization domains permits the chimeric blocking protein monomers to trimerize. While, as monomers, the chimeric blocking proteins can saturate the ligands present on the fibers, such bonds are, of course, subject to dissociation at a certain rate depending on the kinetics of the ligand-substrate interaction. However, because the probability that all three ligand/substrate bonds between a trimeric fiber and the trimeric blocking protein will be severed at the same time is significantly less than the probability that any one such bond will be broken, a trimeric blocking protein more easily saturates the available ligands present on the fiber. In effect, the trimeric structure effectively holds each substrate against the fiber knob ligand, thereby increasing the likelihood that each ligand is blocked.

The chimeric blocking proteins can be produced by any suitable method, such as by direct protein synthesis, cellular production, *in vitro* translation or other method known in the art. Many suitable methods for producing proteins are described elsewhere herein and are otherwise known in the art.

Viruses

The present invention provides an adenovirus incorporating the recombinant fiber trimers of the present invention. The adenovirus of the present invention does not infect its native host cell via the native AR as readily as the wild-type serotype, due to the above-mentioned reduction in affinity of the fiber trimers present in the viral coat (e.g., via replacement of the trimerization domain with a non-ligand trimerization domain, selective mutation of the responsible residues, or incorporation of a blocking domain, as herein described). Thus, the adenovirus preferably incorporates a non-adenoviral ligand to facilitate its propagation, isolation and/or targeting.

The virus can include any suitable ligand (e.g., a peptide specifically binding to a substrate). For example, for targeting the adenovirus to a cell type other than that naturally infected (or a group of cell types other than the natural range or set of host cells), the ligand can bind a cell surface binding site (e.g., any site present on the surface of a cell with which the adenovirus can interact to bind the cell and thereby promote cell entry) other than its native AR or even any native AR. A cell surface binding site can be any suitable type of molecule, but typically is a protein (including a modified protein), a carbohydrate, a glycoprotein, a proteoglycan, a lipid, a mucin molecule or mucoprotein, or other similar molecule. Examples of potential cell surface binding sites include, but

are not limited to: heparin and chondroitin sulfate moieties found on glycosaminoglycans; sialic acid moieties found on mucins, glycoproteins, and gangliosides; common carbohydrate molecules found in membrane glycoproteins, including mannose, N-acetyl-galactosamine, N-acetyl-glucosamine, fucose, and galactose; glycoproteins such as ICAM-1, VCAM, selectins (e.g., E-selectin, P-selectin, L-selectin, etc.), and integrin molecules; and tumor-specific antigens present on cancerous cells, such as, for instance, MUC-1 tumor-specific epitopes. The protein can thus be expressed in a narrow class of cell types (e.g., cardiac muscle, skeletal muscle, smooth muscle, etc.) or expressed within a broader group encompassing several cell types.

In other embodiments (e.g., to facilitate purification or propagation within a specific engineered cell type), the non-native ligand can bind a compound other than a natural cell-surface protein. Thus, the ligand can bind blood- and/or lymph-borne proteins (e.g., albumin), synthetic peptide sequences such as polyamino acids (e.g., polylisine, polyhistadine, etc.), artificial peptide sequences (e.g., FLAG SEQ ID NO:16), and RGD peptide fragments (Pasqualini et al., *J. Cell. Biol.*, *130*, 1189 (1995)). Alternatively, the ligand can bind non-peptide substrates, such as plastic (e.g., Adey et al., *Gene*, *156*, 27 (1995)), biotin (Saggio et al., *Biochem. J.*, *293*, 613 (1993)), a DNA sequence (Cheng et al., *Gene*, *171*, 1, (1996); Krook et al., *Biochem. Biophys., Res. Commun.*, *204*, 849 (1994)), streptavidin (Geibel et al., *Biochemistry*, *34*, 15430 (1995), Katz, *Biochemistry*, *34*, 15421 (1995)), nitrostreptavidin (Balass et al., *Anal. Biochem.*, *243*, 264 (1996)), heparin (Wickham et al., *Nature Biotechnol., 14*, 1570-73 (1996)), cationic supports, metals such as nickel and zinc (e.g., Rebar et al., *Science, 263*, 671 (1994); Qui et al., *Biochemistry, 33*, 8319 (1994)), or other potential substrates. Examples of suitable ligands and their substrates for use in the method of the invention include, but are not limited to: CR2 receptor binding the amino acid residue attachment sequences, CD4 receptor recognizing the V3 loop of HIV gp120, transferrin receptor and its ligand (transferrin), low density lipoprotein receptor and its ligand, the ICAM-1 receptor on epithelial and endothelial cells in lung and its ligand, linear or cyclic peptide ligands for streptavidin or nitrostreptavidin (Katz, *Biochemistry*, *34*, 15421 (1995)), galactin sequences that bind lactose, galactose and other galactose-containing compounds, and asialoglycoproteins that recognize deglycosylated protein ligands. Moreover, additional ligands and their binding sites preferably include (but are not limited to) short (e.g., 6 amino acid or less) linear stretches of amino acids recognized by integrins, as well as polyamino acid sequences such as polylysine, polyarginine, etc. Inserting multiple lysines and/or arginines provides for recognition of heparin and DNA. Also, a ligand can comprise a commonly employed peptide tag (e.g., short amino acid sequences known to be recognized by available antisera) such as sequences from glutathione-S-transferase (GST) from *Shistosoma manosi*, thioredoxin β-galactosidase, or maltose binding protein

(MPB) from *E. coli.*, human alkaline phosphatase, the FLAG octapeptide (SEQ ID NO:16), hemagluttinin (HA) (Wickham et al., 1996, *supra*), polyoma virus peptides, the SV40 large T antigen peptide, BPV peptides, the hepatitis C virus core and envelope E2 peptides and single chain antibodies recognizing them (Chan, *J. Gen. Virol.*, *77*, 2531 (1996)), the c-myc peptide, adenoviral penton base epitopes (Stuart et al., *EMBO J.*, *16*, 1189-98 (1997)), epitopes present in the E2 envelope of the hepatitis C virus SEQ ID NO:17, SEQ ID NO:18 (see, e.g., Chan et al., 1996, *supra*), and other commonly employed tags. A preferred substrate for a tag ligand is an antibody directed against it, a derivative of such an antibody (e.g., a FAB fragment, Single Chain antibody (ScAb)), or other suitable substrate.

As mentioned, a suitable ligand can be specific for any desired substrate, such as those recited herein or otherwise known in the art. However, adenoviral vectors can also be engineered to include novel ligands by first assaying for the ability of a peptide to interact with a given substrate. Generally, a random or semirandom peptide library containing potential ligands can be produced, which is essentially a library within an expression vector system. Such a library can be screened by exposing the expressed proteins (i.e., the putative ligands) to a desired substrate. Positive selective binding of a species within the library to the substrate indicates a ligand for that substrate, at least under the conditions of the assay. For screening such a peptide library, any assay able to detect interactions between proteins and substrates is appropriate, and many are known in the art. However, one preferred assay for screening a protein library is the phage display system, which employs bacteriophage expressing the library (e.g., Koivunen et al., *Bio/Technology*, *13*, 265-70 (1995); Yanofsky et al., *Proc. Nat. Acad. Sci. U.S.A.*, *93*, 7381-86 (1996); Barry et al., *Nature Med.*, *2*(3), 299-305 (1996)). Binding of the phage to the substrate is assayed by exposing the phage to the substrate, rinsing the substrate, and selecting for phage remaining bound to the substrate. Subsequently, limiting dilution of the phage can identify individual clones expressing the putative ligand. Of course, the insert present in such clones can be sequenced to determine the identity of the ligand.

Phage display is preferred for identifying potential ligands because it best mimics viral interaction with the microenvironment. Notably, phage display is an extracellular system (as is the initial stage of viral infection); moreover, phage display incorporates an actual virus (phage) presenting the actual potential ligand. Phage display also offers significantly more flexibility than other protein binding assays (especially intracellular assays). Notably, phage display not only identifies proteins (ligands) binding to a particular substrate, but it identifies those which bind under predefined conditions. Thus, the use of phage display can identify ligands useful for incorporation into an adenovirus to facilitate purification under largely predefined conditions. For example, the phage display library can be screened by exposure to a particular plastic, resin, or other desired

substrate used in an affinity column. Phage expressing peptides that either bind the substrate or that are eluted from the substrate under a specific condition or range of conditions (e.g., high or low salt, pH, temperature, etc.), but do not so bind or elute under other conditions, can be readily identified. Thereafter, adenovirus incorporating the ligand can be purified by expositing it to the substrate under like conditions, as discussed herein.

Once a given ligand is identified, it can be incorporated into any location of the virus capable of interacting with a substrate (i.e., the viral surface). For example, the ligand can be incorporated into the fiber, the penton base, the hexon, or other suitable location. Where the ligand is attached to the fiber protein, preferably it does not disturb the interaction between viral proteins or monomers. Thus, the ligand preferably is not itself an oligomerization domain, as such can adversely interact with the trimerization domain as discussed above. Moreover, the ligand preferably does not replace a portion of the fiber protein, as such perturbance can adversely affect trimerization and interaction with the penton. Rather, the ligand preferably is added to the fiber protein, and is incorporated in such a manner as to be readily exposed to the substrate (e.g., at the carboxy-terminus of the protein, attached to a residue facing the substrate, positioned on a peptide spacer to contact the substrate, etc.) to maximally present the ligand to the substrate. Where the ligand is attached to or replaces a portion of the penton, preferably it is within the hypervariable regions to ensure that it contacts the substrate. Furthermore, where the ligand is attached to the penton, preferably, the recombinant fiber is truncated or short (e.g., from 0 to about 10 shaft repeats) to maximally present the ligand to the substrate (see, e.g., U.S. Patent 5,559,099 (Wickham et al.)). Where the ligand is attached to the hexon, preferably it is within a hypervariable region (Miksza et al., *J. Virol.*, *70*(3), 1836-44 (1996)).

When engineered into an adenoviral protein (or blocking protein), the ligand can comprise a portion of the native sequence in part and a portion of the non-native sequence in part. Similarly, the sequences (either native and/or nonnative) that comprise the ligand in the protein need not necessarily be contiguous in the chain of amino acids that comprise the protein. In other words, the ligand can be generated by the particular conformation of the protein, e.g., through folding of the protein in such a way as to bring contiguous and/or noncontiguous sequences into mutual proximity. Of course an adenovirus of the present invention (or a blocking protein) can comprise multiple ligands, each binding to a different substrate. For example, a virus can comprise a first ligand permitting affinity purification as described herein, a second ligand that selectively binds a cell-surface site as described herein, and/or a third ligand for inactivating the virus, also as described herein.

The protein including the ligand can include other non-native elements as well. For example, a non-native, unique protease site also can be inserted into the amino acid sequence. The protease site preferably does not affect fiber trimerization or substrate specificity of the fiber ligand. Many such protease sites are known in the art. For example, thrombin recognizes and cleaves at a known amino acid sequence (Stenflo et al., *J. Biol. Chem.*, *257*, 12280-90 (1982)). The presence of such a protease recognition sequence facilitates purification of the virus in some protocols, as discussed herein. The protein can be engineered to include the ligand by any suitable method, such as those methods described above for introducing mutations into proteins.

In addition to the trimer and the ligand, a virus of the present invention can include one or more non-native passenger genes as well. A "passenger gene"" can be any suitable gene, and desirably is either a therapeutic gene (i.e., a nucleic acid sequence encoding a product that effects a biological, preferably a therapeutic, response either at the cellular level or systemically), or a reporter gene (i.e., a nucleic acid sequence which encodes a product that, in some fashion, can be detected in a cell). Preferably a passenger gene is capable of being expressed in a cell into which the vector has been internalized. Preferably the passenger gene exerts its effect at the level of RNA or protein. For instance, a protein encoded by a transferred therapeutic gene can be employed in the treatment of an inherited disease, such as, e.g., the cystic fibrosis transmembrane conductance regulator cDNA for the treatment of cystic fibrosis. Alternatively, the protein encoded by the therapeutic gene can exert its therapeutic effect by effecting cell death. For instance, expression of the gene in itself can lead to cell killing, as with expression of the diphtheria toxin. Alternatively, a gene, or the expression of the gene, can render cells selectively sensitive to the killing action of certain drugs, e.g., expression of the HSV thymidine kinase gene renders cells sensitive to antiviral compounds including aciclovir, ganciclovir, and FIAU (1-(2-deoxy-2-fluoro-β-D-arabinofuranosil)-5-iodouracil). Moreover, the therapeutic gene can exert its effect at the level of RNA, for instance, by encoding an antisense message or ribozyme, a protein which affects splicing or 3' processing (e.g., polyadenylation), or a protein affecting the level of expression of another gene within the cell (i.e., where gene expression is broadly considered to include all steps from initiation of transcription through production of a processed protein), perhaps, among other things, by mediating an altered rate of mRNA accumulation, an alteration of mRNA transport, and/or a change in post-transcriptional regulation. Of course, where it is desired to employ gene transfer technology to deliver a given passenger gene, its sequence will be known in the art.

The altered protein (e.g., the trimer or the coat protein having the ligand) and the passenger gene (where present) can be incorporated into the adenovirus by any suitable method, many of which are known in the art. As mentioned herein, the protein is

preferably identified by assaying products produced in high volume from genes within expression vectors (e.g., baculovirus vectors). The genes from the vectors harboring the desired mutation can be readily subcloned into plasmids, which are then transfected into suitable packaging cells (e.g., 293 cells). Transfected cells are then incubated with adenoviruses under conditions suitable for infection. At some frequency within the cells, homologous recombination between the vector and the virus will produce an adenoviral genome harboring the desired mutation.

Adenoviruses of the present invention can be either replication competent or replication deficient. Preferably, the adenoviral vector comprises a genome with at least one modification therein, rendering the virus replication deficient (see, e.g., International Patent Application WO 95/34671). The modification to the adenoviral genome includes, but is not limited to, addition of a DNA segment, rearrangement of a DNA segment, deletion of a DNA segment, replacement of a DNA segment, or introduction of a DNA lesion. A DNA segment can be as small as one nucleotide and as large as the adenoviral genome (e.g., about 36 kb) or, alternately, can equal the maximum amount which can be packaged into an adenoviral virion (i.e., about 38 kb). Preferred modifications to the adenoviral genome include modifications in the E1, E2, E3, and/or E4 regions. An adenovirus also preferably can be a cointegrate, i.e., a ligation of adenoviral genomic sequences with other sequences, such as other virus, phage, or plasmid sequences.

The adenovirus of the present invention has many qualities which render it an attractive choice for use in gene transfer, as well as other, applications. For example, the adenovirus does not infect its native host cells as readily as does wild-type adenovirus, due to the mutant fiber trimers (e.g., selective mutation of residues responsible for AR binding, replacement of the trimerization domain, or addition of a blocking domain, as herein described). Furthermore, the adenovirus has at least one non-native ligand specific for a substrate which facilitates viral propagation, targeting, purification, and/or inactivation as discussed herein. For ease in cloning, the ligands and the trimerization domains preferably are separate domains, thus permitting the virus to be easily be reengineered to incorporate different ligands without perturbing fiber trimerization. Alternatively, if the fiber trimer incorporates a mutated fiber knob, the ligand can be incorporated into the knob, as herein described.

Of course, for delivery into a host (such as an animal), a virus of the present invention can be incorporated into a suitable carrier. As such, the present invention provides a composition comprising an adenovirus of the present invention and a pharmacologically acceptable carrier. Any suitable preparation is within the scope of the invention, the exact formulation, of course, depends on the nature of the desired application (e.g., cell type, mode of administration, etc.), many suitable preparations are set forth in U.S. Patent 5,559,099 (Wickham et al.).

Cell Line

As mentioned herein, an adenovirus of the present invention does not readily infect its native host cell via the native AR because its ability to bind ARs is significantly attenuated (due to the incorporation of the chimeric trimers of the present invention). Therefore, the invention provides a cell line able to propagate the inventive adenovirus. Preferably, the cell line can support viral growth for at least about 10 passages (e.g., about 15 passages), and more preferably for at least about 20 passages (e.g., about 25 passages), or even 30 or more passages.

For example, the adenoviruses can be first grown in a packaging cell line which expresses a native fiber protein gene. The resultant viral particles are therefore likely to contain both native fibers encoded by the complementing cell line and non-native fibers encoded by the adenoviral genome (such as those fibers described herein); hence a population of such resultant viruses will contain both fiber types. Such particles will be able to bind and enter packaging cell lines via the native fiber more efficiently than particles which lack native fiber molecules. Thus, the employment of such a fiber-encoding cell line permits adenovirus genomes encoding chimeric, targeted adenovirus fibers to be grown and amplified to suitably high titers. The resultant "mixed" stocks of adenovirus produced from the cell lines encoding the native fiber molecule will contain both native and chimeric adenovirus fiber molecules; however, the particles contain genomes encoding only the chimeric adenovirus fiber. Thus, to produce a pure stock of adenoviruses having only the chimeric adenovirus fiber molecules, the "mixed" stock is used to infect a packaging cell line which does not produce native fiber (such as 293 for E1-deleted viruses). The resultant adenoviruses contain only the fiber molecules encoded by the genomes (i.e., the chimeric fiber molecules).

Similar fiber-complementing cell lines have been produced and used to grow mutant adenovirus lacking the fiber gene. However, the production rates of these cell lines have generally not been great enough to produce adenovirus titers of the fiber-deleted adenovirus comparable to those of fiber-expressing adenovirus particles. The lower titers produced by such mutants can be improved by temporally regulating the expression of the native fiber to more fully complement the mutant adenovirus genome. One strategy to produce such an improved cell line is to use of an inducible promoter, (e.g., the metallothionine promoter), to permit fiber production to be controlled and activated once the cells are infected with adenovirus. Alternatively, an efficient mRNA splice site introduced into the fiber gene in the complementing cell line improves the level of fiber protein production in the cell line.

When the adenovirus is engineered to contain a ligand specific for a given cell surface binding site, any cell line expressing that receptor and capable of supporting

adenoviral growth is a suitable host cell line. However, because many ligands do not bind cell surface binding sites (especially the novel ligands discussed herein), a cell line can be engineered to express the substrate for the ligand.

The present invention provides a cell line expressing a non-native cell-surface biding site to which an adenovirus (or a bi-specific blocking protein) having a ligand for the receptor binds. Any cell line capable of supporting adenoviral growth is a suitable cell line for use in the present invention. Where the adenovirus lacks genes essential for viral replication, preferably the cell line expresses complementing levels of the gene products. As 293 cells are superior for supporting adenoviral growth, preferably the cell line of the present invention is derived from 293 cells.

The non-native cell surface binding site is a substrate molecule, such as those described herein, to which an adenovirus (or a bi-specific blocking protein) having a ligand selectively binding that substrate can bind the cell and thereby promote cell entry. Where the ligand is on the adenovirus, the binding site can recognize a non-native ligand incorporated into the adenoviral coat or a ligand native to a virus. For example, where the non-native viral ligand is a tag peptide, the binding site can be a single chain antibody (ScAb) receptor recognizing the tag. Alternatively, the ScAb can recognize an epitope present in a region of a mutated fiber knob (where present), or even an epitope present on a native adenoviral coat protein, (e.g., on the fiber, penton, hexon, etc.). Alternatively, where the non-native ligand recognizes a cell-surface substrate (e.g., membrane-bound protein), the binding site can comprise that substrate. Where the substrate binding side is native to a cell-surface receptor, the cell line can express a mutant receptor with decreased ability to interact with the cellular signal transduction pathway (e.g., a truncated receptor, such as NMDA, (Li, et al., *Nat. Biotech.*, *14*, 989 (1996)), attenuated ability to act as an ion channel, or other modification. Infection via such modified proteins minimizes the secondary effects of viral infection on host-cell metabolism by reducing the activation of intracellular messaging pathways and their various response elements. In short, the choice of binding site depends to a large extent on the nature of the adenovirus in question. However, to promote specificity of the cell type for the virus, the binding site preferably is not a native mammalian AR. Moreover, the binding site must be expressed on the surface of the cell to be accessible to the virus. Hence, where the binding site is a protein, it preferably has leader sequence and a membrane tethering sequence (see, e.g., Davitz et al., *J. Exp. Med. 163*, 1150 (1986)). to promote proper integration into the membrane.

The cell line can be produced by any standard method. For example, a vector (e.g., an oligonucleotide, plasmid, viral, or other vector) containing a gene encoding the non-native receptor can be introduced into source cell line by standard means. Preferably, the vector also encodes an agent permitting the cells harboring it to be selected (e.g., the

vector can encode resistance to antibiotics which kill cells not harboring the plasmid). At some frequency, the vector will recombine with the cell genome to produce a transformed cell line expressing the binding site.

Method of Propagation

In connection with the cell line expressing a non-native adenoviral cell-surface binding site, the present invention provides a method of propagating the inventive adenovirus. The inventive method involves infecting the cell with an adenovirus having a non-native ligand selectively binding to the receptor, incubating the cells, and recovering the adenoviruses produced within the cells. Adenoviruses recovered from the cells can be propagated again (e.g., amplified) to produce viral stocks of very high titer. The ligand on the adenovirus can be any ligand, such as those discussed herein. The cells of the present invention are infected by the virus at any suitable m.o.i. to promote efficient infection of the cell line (e.g., from about 1 m.o.i. to about 10 m.o.i.). The conditions of cell culture largely depend on the nature of the host cell. However, it is within the skill of the art to select culture conditions suitable for a given cell type. Viruses are recovered from the cells by standard means, such as by cell lysis. Thereafter they can be purified by standard methods or the method of the present invention.

Method of Purifying

As mentioned, the substrate for the ligand engineered into the adenovirus need not be present on the surface of a cell. For example, the substrate can be located on a support, e.g., an inanimate support such as plastic, glass, metal, resin, or other material commonly employed in chromatographic or affinity separation. Examples of such supports include metals, natural polymeric carbohydrates and their synthetically modified, cross-linked or substituted derivatives, such as agar, agarose, cross-linked alginic acid, substituted and cross-linked guar gums, cellulose esters, especially with nitric acid and carboxylic acids, mixed cellulose esters, and cellulose ethers; natural polymers containing nitrogen, such as proteins and derivatives, including cross-linked or modified gelatins; natural hydrocarbon polymers, such as latex and rubber; synthetic polymers which may be prepared with suitably porous structures, such as vinyl polymers, including polyethylene, polypropylene, polystyrene, polyvinylchloride, polyvinylacetate and its partially hydrolyzed derivatives, polyacrylamides, polymethacrylates, copolymers and terpolymers of the above polycondensates, such as polyesters, polyamides, and other polymers, such as polyurethanes or polyepoxides; porous inorganic materials such as sulfates or carbonates of alkaline earth metals and magnesium, including barium sulfate, calcium sulfate, calcium carbonate, silicates of alkali and alkaline earth metals, aluminum and magnesium; and aluminum or silicon oxides or hydrates, such as clays, alumina, talc, kaolin, zeolite,

silica gel, or glass (these materials may be used as filters with the above polymeric materials); and mixtures or copolymers of the above classes, such as graft copolymers other material commonly employed in chromatographic or affinity separation. Such supports can be fashioned into beads, films, sheets, plates, etc., or coated onto, bonded, laminated, or otherwise joined to appropriate inert carriers, such as paper, glass, polymeric films, fabrics, etc.

The presence of a substrate for a ligand on the surface of an adenovirus of the present invention permits adenoviruses to be readily purified with high affinity and fidelity. Accordingly, the present invention provides a method of purifying an adenovirus having a ligand for a substrate from a composition comprising the adenovirus. The method involves exposing the composition to the substrate under conditions to promote the ligand present on the adenovirus to selectively bind the substrate. Subsequently, the composition (e.g., at least a significant portion of the composition) not selectively binding the substrate is removed from the substrate, after which the adenovirus bound to the substrate is eluted from the substrate. Using this method, an adenovirus having a ligand can be purified from a variety of compositions (e.g., solutions, dispersions, suspensions, gels, etc.). While adenoviruses can be present in a variety of compositions, a common composition containing adenoviruses is a cell lysate, such as produced from a packaging cell during adenoviral propagation.

Generally, the substrate is bound to a support, as previously described. Fusing desired ligand-substrates to a suitable support material is known in the art, and the present invention contemplates any suitable method for engineering a support having the substrate. Indeed, as mentioned, the substrate can itself be such a plastic, glass, metal, resin, etc. Any method of exposing the composition containing the adenovirus to the substrate is suitable for use in the present inventive method. For example, the composition can be passed through a column comprising the support onto which the substrate is bound. Of course, the composition also can be mixed with a slurry of such a support (e.g., beads or other preparation comprising the support-bound substrate), placed into a container (e.g., a tube, the well of a dish, etc.) which has been coated with the substrate, or otherwise exposed to the substrate.

The parameters of time, temperature, and solution chemistry necessary to promote selective binding can vary according to the affinity with which the ligand selectively binds the substrate. Generally, where known ligand-substrate systems are employed, these parameters are also known. Where novel ligand-substrate systems are employed, however, the binding conditions can, in large measure, be predetermined as discussed herein (e.g., by employing such conditions when screening the protein library for the novel ligand-substrate interaction). Preferably, the conditions for selective binding do not permit selective binding of other constituents of the composition to the substrate. Where

other constituents do not selectively bind the substrate, a significant amount of the adenovirus can be removed from the composition by association with the substrate.

After the selective binding step, the adenoviral-deprived composition is removed from the presence of the substrate (e.g., selectively eluted). Any suitable method for so removing the adenoviral-deprived composition from the substrate can be employed, provided the adenovirus remains selectively bound to the substrate. In other words, the conditions employed for removing the adenoviral-deprived composition from the substrate generally are insufficient to elute the adenovirus from the substrate. The method of removing the adenoviral-deprived composition is largely a function of the type of substrate and support. For example, the adenoviral-deprived composition can be removed from a column comprising the substrate by rinsing the column with several volumes of a suitable solution. Moreover, the adenoviral-deprived composition can be removed from a slurry of the support containing the substrate by repeated centrifugation, resuspension in a suitable solution, and recentrifugation. Alternatively, where the support is a magnetic material, it can be physically removed from the solution by exposing the vessel containing the solution to a magnet and rinsing the magnetic support. Moreover, where the substrate is bound to a dish or a well, the dish can simply be rinsed with several volumes of a suitable solution.

After the adenoviral-deprived composition has been removed from the substrate, the adenovirus is eluted from the substrate. Any method for separating the adenovirus from the substrate is suitable for use in the present inventive method. In many applications, the adenovirus can be liberated by exposing the support-adenovirus complex to an elution solution incompatible with the ligand-substrate bond. The parameters of time, temperature, and solution chemistry necessary to promote selective elution of the virus from the support can vary according to the affinity with which the ligand selectively binds the substrate. Generally, where known ligand-substrate systems are employed, these parameters are also known. Where novel ligand-substrate systems are employed, however, the elution conditions can, in large measure, be predetermined, for example, by adjusting the conditions when screening a protein library, as discussed herein. Additionally, where the ligand is incorporated into the adenovirus on a spacer or other peptide, as described, the spacer can include a peptidase recognition sequence or other specific cleavage motif. Adenoviruses containing such a cleavage sequence can be liberated from the support by exposing the support to an agent effecting the cleavage, such as an endoprotease or other agent. While the cleavage method severs the ligand from the adenovirus, in many applications this is preferred. For example, the ligand for purifying the virus might interfere with a second ligand for targeting the virus to a particular cell type. Removal of the purifying ligand thus permits the isolated adenovirus to more readily infect the cell type of interest.

While any suitable binding or elution conditions can be employed, a practical limit is set by the ability of the adenovirus to survive the conditions. However, as adenoviruses are able to withstand a wide variety of environmental variation, such as high salt, high osmolality, and basic conditions, the present method can be employed under a wide range of conditions. In any event, such conditions are known to those of skill in the art.

The inventive method for purifying adenoviruses need not remove all of the virus from the solution, or even a majority of the virus. Indeed, in many applications, the amount of virus present in the initial composition can saturate the amount of substrate present on the support. Moreover, while the ligand on the adenovirus selectively binds the substrate, such selective binding can be of any affinity. As such, a substantial amount of substrate can not bind available ligands in the separation step. Therefore, to obtain as much adenovirus from the initial composition as possible, the adenoviral-depleted composition removed from the support, as herein described, can be subjected to successive rounds of purification, and the viruses obtained from each round can be combined into a single stock. Similarly, while other constituents of the initial composition preferably do not selectively bind the resin, the complete absence of erroneous binding is not common, at least in early rounds of purification. The presence of background levels of erroneous binding necessarily results in some contamination of the initial viral stock obtained. To reduce or substantially eliminate such background contamination, the viral stock can be subjected to successive rounds of purification until the background level of contaminants approaches zero. As such, the present inventive method provides an economical, efficient, and reliable means of purifying adenoviruses having known ligands. Moreover, the use of slurries and columns is common in industrial applications, rendering the present method amenable to high throughput, or commercial-scale application.

Method of Infecting a Cell

As mentioned, the non-native ligand present on the virus of the present invention (or on the virus/blocking protein complex) can recognize a substrate present within a cell surface binding site. Therefore, the present invention provides a method of infecting a cell having a cell surface binding site including a substrate for the non-native ligand. The method involves contacting the cell with the adenovirus such that the non-native ligand of the adenovirus (or on the virus/blocking protein complex) binds the particular cell surface binding site and thereby effects entry of the adenovirus. Because the viruses of the present invention incorporate fiber trimers having reduced ability to bind native mammalian ARs, the adenovirus is internalized into the cell primarily due to the non-native ligand. As such, the present inventive method effects selective targeting of the virus comprising the ligand to a cell type expressing a binding site comprising the

substrate for that ligand without significant infection of cells via native mammalian ARs. In the case where the ligand is on the penton base (such as a modified or unmodified penton base), the virus is internalized via the ligand on the penton.

Any cell expressing a cell surface binding site including a substrate for the ligand can be selectively targeted in accordance with the present invention. A cell can be present as a single entity, or can be part of a larger collection of cells, such as a cell culture (either mixed or pure), a tumor, a tissue (e.g., epithelial, muscle, or other tissue), an organ, an organ system (e.g., circulatory system, respiratory system, gastrointestinal system, urinary system, nervous system, integumentary system or other organ system), or even an entire organism (e.g., a human). Preferably, the cells being targeted are selected from the group consisting of heart, blood vessel, smooth muscle, skeletal muscle, lung, liver, gallbladder, urinary bladder, and eye cells.

The method for infecting a cell ideally is carried out wherein the adenovirus includes a passenger gene, such as those vectors herein described. Where the adenovirus of the present invention includes a passenger gene, the method permits the adenovirus to serve as a vector for introducing that gene into a targeted cell. Once internalized, the passenger gene is expressed within the cell. Thus, the vectors and methods of the present invention provide useful tools for introducing a passenger gene into a selected class of cells without significantly providing the gene to cells ubiquitously or ectopicly.

Method of Inactivating a Virus

As mentioned, the non-native ligand present on the virus of the present invention can recognize substrate present within blood or lymphatic fluid (such as a ligand present on a free blood-borne protein, a protein present on erythrocytes, etc.). Therefore, the present invention provides a method of inactivating an adenovirus having a ligand recognizing a blood- or lymph-borne substrate by exposing the virus to the substrate. Within the blood or lymph, the ligand selectively binds its substrate, thereby adsorbing the free virus from the fluid. Preferably, the substrate is present within a large macromolecule (e.g., albumin) or on the surface of erythrocytes (which lack transcription machinery required to propagate viruses). Of course, a ligand for inactivating the virus can be present at any location on the viral coat (Fender et al., *Virology*, *214*, 110 (1995)). However, as antibodies recognizing and/or neutralizing adenoviruses primarily bind epitopes on the hexon (Gahery-Segard et al., *Eur. J. Immunol.*, *27*, 653 (1997)), non-native ligands for inactivation of the virus preferably are incorporated into the hexon, as herein described.

By providing a means of effectively inactivating adenoviruses, the method assists in confining the viral infection to a desired locus (tissue, cell type, etc.). Specifically, the method effectively inactivates an individual virus by tethering it to the substrate, thereby

reducing its ability to contact (and therefore enter) a cell. Even where a virus so adsorbed does contact a cell, it is significantly less likely to be internalized due to the presence of the particle having the substrate. Due to the aggregation of these effects, the inventive method effectively inactivates a viral stock (outside of the desired locus of infection) by dramatically reducing its effective free titer.

The inventive method for inactivating the virus complements the other embodiments of the present invention. For example, as stated, the viruses of the present invention incorporate fiber trimers having reduced affinity for native mammalian ARs, thereby substantially reducing the likelihood that the virus will infect cell types other than the desired cell type. Moreover, the viruses of the present invention can include ligands specific for a substrate present on a cell surface binding site, permitting the virus to be targeted to a predetermined cell type. While those two qualities effect selective targeting, and thereby significantly attenuate ectopic infection, viruses also having a ligand recognizing a blood- or lymph- borne substrate are much less likely to even contact an ectopic tissue by reason of the effective reduction of viral titer.

While it is believed that one of skill in the art is fully able to practice the invention after reading the foregoing description, the following examples further illustrate some of its features. As these examples are included for purely illustrative purposes, they should not be construed to limit the scope of the invention in any respect. The procedures employed in these examples, such as affinity chromatography, Southern blots, PCR, DNA sequencing, vector construction (including DNA extraction, isolation, restriction digestion, ligation, etc.), cell culture (including antibiotic selection), transfection of cells, protein assays (Western blotting, immunoprecipitation, immunofluorescence), etc., are techniques routinely performed by those of skill in the art (see generally Sambrook et al., *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)). Accordingly, in the interest of brevity, experimental protocols are not discussed in detail.

EXAMPLE 1

This example describes two different fiber trimers having non-native trimerization domains, each of which interacts properly with the adenoviral penton base. Specifically, the fiber chimeras incorporate the reovirus sigma 1 trimerization domain.

Two chimeras were constructed, T5S7sigDel and T5sigDel. T5sigDel contained only the Ad5 fiber tail (T5) fused to sigDel without any Ad fiber shaft sequence. T5S7sigDel contained the tail plus the first 7 β-sheet repeats of the Ad shaft (S7) fused to sigDel. The DNA and respective amino acid sequences of these two clones are set forth at SEQ ID NO:1 and SEQ ID NO:2.

The sigDel region of the reovirus sigma 1 gene was amplified via PCR and cloned into the vector, pAcT5S7GCNTS.PS.LS.X (Fig. 3A), to create the baculovirus transfer vector, pAcT5sigDel.TS.PS.LS (Fig. 3B). This vector encodes the Ad5 fiber tail fused to the N-terminal trimerization domain of reovirus type 3 sigma 1 protein followed by a FLAG epitope near the C-terminus. At the C-terminus of the gene, the vector also contains multiple restriction sites to facilitate the cloning of targeting and purification sequences into the gene.

The second vector, pAcT5S7sigDel.TS.PS.LS (Fig. 3C), was created by cutting the above PCR product with the restriction enzymes NheI and BamHI and cloning this fragment into the vector, pAcT5S7GCNTS.PS.LS.X (Fig. 3A), also cut with NheI and BamHI. The resultant vector encodes a protein containing the tail and first seven β-sheet shaft repeats of Ad5 fiber fused to sigDel, followed by a FLAG epitope.

Recombinant baculovirus clones encoding each of the fiber chimeras were then generated by standard means using each of the above plasmids. The resultant baculovirus clones were used to produce recombinant proteins in Tn5 insect cells. To compare the sigDel trimerization domain with the GCN domain, another baculovirus was constructed from the initial plasmid, pAcT5S7GCNTS.PS.LS.X (Fig. 3A), which contained the GCN trimerization domain in place of the sigDel trimerization domain.

The baculovirus-infected cells were pelleted at 3 days post infection. The cell pellet was resuspended in PBS plus protease inhibitors and freeze-thawed three times to release the soluble intracellular proteins. The cell debris were then pelleted by centrifugation at high speed and the cleared cell lysate was removed. The pellet was then resuspended in the same volume of PBS as previously.

Pellet and lysate samples were then run on an 0.1% SDS, 12.5% polyacrylamide gel and transferred to nitrocellulose for Western analysis using anti-FLAG M2 MAb (Kodak). These results demonstrated that over 90% of each of the proteins, T5S7GCN.TS.PS.LS, T5sigDel.TS.PS.LS and T5S7sigDel.TS.PS.LS were soluble in the lysate.

The proteins were further assayed for their ability to form trimers. To test for chimera trimerization, the lysates from each sample were either boiled or not boiled prior to running the samples on a 0.1% SDS, 12.5% polyacrylamide gel. Western analysis of the boiled samples showed that the boiled samples migrated at molecular weights corresponding to the size of the monomeric protein, whereas the unboiled proteins containing the sigDel trimerization domains migrated at molecular weights commensurate with a trimer. The unboiled T5S7GCN.TS.PS.LS protein also migrated as a trimer; however, a significant portion (over half) of the unboiled sample migrated as a monomer. Similar analyses of wild type fiber and sigma 1 protein have shown that these proteins migrate completely as trimers when not boiled and as monomers when boiled.

That the vast majority of the proteins were soluble in the lysate (as opposed to the pellet) strongly suggests that they were correctly folded. Moreover, the migration of the unboiled samples demonstrates that sigDel-containing chimeras are soluble trimers and that the sigDel domain functions better than GCN by forming more stable trimeric fiber chimeras.

To test for the ability of the trimers to complex properly with adenoviral penton base protein, recombinant penton base is mixed in solution with the T5S7GCN.TS.PS.LS, T5sigDel.TS.PS.LS and T5S7sigDel.TS.PS.LS trimeric fiber proteins. The resultant penton base/fiber chimera complex is then immunoprecipitated with anti-penton base antibody coupled to protein A-agarose. The precipitated sample is then run on an SDS-PAGE gel and evaluated by Western analysis as described above using the FLAG antibody. Binding of the FLAG antibody indicates that the fiber chimera containing the FLAG epitope complexes with the penton base in solution.

EXAMPLE 2

This example demonstrates the ability of the fiber-sigDel chimeras to incorporate exogenous protein domains larger than peptide tags.

The sequence encoding a modified version of the green fluorescent protein was amplified by PCR using the primers containing restriction sites to allow efficient cloning into the fiber-sigDel chimera plasmids described above in Example 1. Cloning of the GFP sequence in the proper orientation into the SpeI site of pAcT5sigDel.TS.PS.LS (Fig. 3B) yields the plasmid, pAcT5sigDel.GFP.TS.PS.LS (Fig. 4), encoding a fiber-sigDel-GFP chimera. The DNA and amino acid sequence of this clone is set forth as SEQ ID NO:3.

This plasmid was then used to produce recombinant protein using the baculovirus expression system, as described above. The solubility of the chimeric fiber proteins (indicative of correct folding) and the ability of the resultant proteins to bind penton base (indicative of trimerization) was confirmed as discussed above. Production of soluble, trimeric protein containing the GFP domains indicates that large, functional protein domains can be incorporated into the fiber-sigDel chimeras as easily as can be the smaller peptide tags. The results predict that such chimeras could also incorporate ligands, such as ScAbs, without significantly interfering with protein function.

EXAMPLE 3

This example describes the construction of recombinant adenovirus vectors containing fiber trimers having non-native trimerization domains.

The NdeI to BamHI fragment is excised from pAcT5S7sigDel.TS.PS.LS (Fig. 3C), to replace the corresponding fragments in pAS pGS HAAV (Fig. 5A), and pAS pGS

pK7 (Fig. 5B), to produce the final transfer vectors pAS T5S7sigDelpGS.HAAV (Fig. 5C) and pAST5S7sigDelpGS.pK7 (Fig. 5D), respectively. The vectors encode the fiber-sigDel chimera containing either the RGD or pK7 binding domains at their C-terminus for binding to an a_v integrin and heparin sulfate-containing receptors that are expressed by 293 cells.

These vectors are then linearized and then transfected 293 cells had been preincubated with the E1, E3, E4-deleted adenovirus AdCMVZ.11A (GenVec, Inc., Rockville, MD) prior to transfection with the plasmids. Recombination of the E4+ pNS plasmid with the E4-deleted vector results in the rescue of an E1-, E3-, E4+ vector capable of replication in 293 cells. The infected/transfected cells are harvested after 5 days and lysed to release virions. The lysate is then used to infect freshly plated cells and to further plaque-purify the recombinant viruses. Plaques cross-contaminated with the original AdCMVZ.11A stain blue when plaqued in medium containing X-glu substrate. White plaques (indicating viable vector) are then amplified to produce pure virus stocks of the recombinant adenovirus.

EXAMPLE 4

This example describes the production of targeted adenovirus particles having genomes encoding chimeric fibers. The chimeric fibers represent the Ad5 fiber tail and seven shaft repeats fused to the sigDel trimerization domain from reovirus followed by a high affinity RGD sequence for binding a_V integrins.

The plasmid, pAS T5S7sigDel.HAAV (Fig. 5C), is cut with the restriction enzyme DrdI, and the large fragment containing all the adenovirus sequences is isolated and purified. This fragment is then electroporated into BJ5183 bacterial cells along with a linearized plasmid, containing the majority of Ad genome prior to the fiber gene with a small overlap of identical sequence with the pAS T5S7sigDel.HAAV plasmid. Upon recombination of the two pieces of DNA, a new plasmid is produced in the bacterial cells through homologous recombination. This plasmid encodes a modified adenovirus genome that is capable of replicating in the appropriate complementing mammalian cell line (E1 and fiber-complementing). The plasmid DNA from selected colonies is isolated and confirmed to be the correct plasmid by restriction analysis. This plasmid DNA is then used to transform DH5a bacterial cells in order to obtain adequate amounts of DNA for transfection into the fiber-complementing cell line.

One microgram of the plasmid is cut with the appropriate restriction enzyme and transfected into a fiber-complementing cell line, such as the cell line described above. At 0-4 days post-transfection, the cells are induced with zinc, and 1-5 days later the cells are lysed. The lysate is passaged onto fresh fiber-complementing cells. This passage and lysis cycle is repeated until a cytopathic effect develops in the cells. During the cycle, the LacZ

activity of the cell lysate is also followed, as it should increase as the recombinant vector is amplified. Once an adequate titer of the "mixed" stock is obtained, a final passage onto non-fiber-complementing cells is made to produce a targeted virus lacking a native fiber protein. The resulting virus is then assayed for its ability to bind and enter cells via the interaction of its high affinity RGD sequence with a_V integrins.

EXAMPLE 5

This example describes four different fiber trimers having non-native trimerization domains. Specifically, the exemplified fiber trimers are chimeras incorporating the knob portion of the NADC-1 fiber, a porcine adenoidal strain. The exemplified trimers, thus, contain known receptor-binding motifs (i.e., a galectin motif and an RGD motif). Furthermore, exemplified trimers incorporate mutations known to reduce the affinity of each of the receptor-binding motifs. Finally, this example describes the incorporation of a non-native ligand (FLAG) into an exposed loop of a non-native trimer.

Using PCR, the knob of the NADC-1 fiber gene was amplified from a plasmid containing the full length gene. The PCR product was then cloned into a baculovirus expression plasmid to produce a plasmid which encoded the NADC-1 knob plus an N-terminal polyhistidine tag (the Pig4KN protein) for purification and detection by Western analysis using an anti-polyhistidine antibody. The DNA and amino acid sequences of this clone are set forth at SEQ ID NO:4.

The resultant plasmid, pAcPig4KN (Fig. 6A), was then mutated by site-directed mutagenesis using the two oligonucleotide primer pairs PigD363Es (SEQ ID NO:10) and PigD363Ea (SEQ ID NO:11), and PigN437Ds (SEQ ID NO:12) and PigN437Da (SEQ ID NO:13). The former pair of primers was used to produce the plasmid pAcPigKN D363E (Fig. 6B), in which the DNA sequence encoding the RGD integrin binding motif (a.a. 361-363 in the native fiber protein) was mutated to the non-functional sequence RGE. The second pair of primers was used to produce the plasmid pAcPigKN N437D (FIG. 6C), in which the DNA sequence encoding the native amino acid N (a.a. 437) was mutated to a D. This mutation has been previously shown to abrogate the binding of another galectin protein to its ligand, galactose (Hirabayashi et al., *J. Biol. Chem., 266*, 23648-53 (1991)).

A final baculovirus plasmid was constructed to demonstrate the feasibility of incorporating a novel binding motif into an exposed loop on the NADC-1 knob. Hydrophobicity analysis of the NADC-1 knob protein revealed that the protein sequence immediately prior to the RGD motif was likely to be an exposed loop that would be capable of incorporating additional amino acid sequences (e.g., polypeptide domains) for the purpose of targeting or purification. Therefore, the plasmid, pAcPig4KN(FLAG) (Fig. 6D), was produced using complementary overlapping oligonucleotides, which

encoded the FLAG binding domain. The oligonucleotides were annealed and cloned into the plasmid pAcPig4KN (Fig. 11A), which contained a unique, native restriction site, AvrII, just prior to sequence encoding the RGD domain.

The four baculovirus transfer plasmids described above carrying NADC-1 knob genes were used to express recombinant protein in insect cells using the baculovirus expression system. Tn5 insect cells were infected with the recombinant baculovirus clones derived from the plasmids. After three days the cells were pelleted and freeze-thawed three times in PBS plus protease inhibitors to release the soluble intracellular protein. The debris were pelleted and the cleared lysate was decanted. The remaining pellet was resuspended in PBS.

Lysate and pellet samples were then evaluated by SDS-PAGE and Western analysis to determine whether the recombinant knob proteins were soluble. Western analysis revealed that the majority of all four knob proteins were present in the cell lysate, indicating that they were soluble and correctly folded. These results demonstrate that neither the point mutations introduced into the receptor-binding domains nor the FLAG binding sequence inserted into an exposed loop adversely affected knob folding and solubility.

To investigate whether the chimeric trimers having the NADC-1 knob-FLAG domains can interact with the FLAG antibody, cell lysates are immunoprecipitated using anti-FLAG M2 antibody and then blotted. Western analysis will demonstrate that the NADC-1 knob containing the FLAG epitope is precipitated by the anti-FLAG antibody. Thus, the NADC-1-fiber trimers are soluble, and each is capable of interacting with the anti-FLAG M2 monoclonal antibody.

EXAMPLE 6

This example describes the synthesis of recombinant Ad5-based vector containing an NADC-1 (porcine adenovirus) fiber knob.

Using PCR, the knob of the NADC-1 fiber gene was amplified from a plasmid containing the full length gene. The PCR product was then cloned into the plasmid PNS F5F2K (Fig. 7A) to produce the plasmid, pNS Pig4.SS (Fig. 7B) which encodes the first 7 β-repeats of the Ad5 shaft fused to the NADC-1 knob. The DNA and amino-acid sequences of this clone are set forth at SEQ ID NO:5.

The pNS Pig4.SS plasmid was then used to create a recombinant adenovirus vector. The plasmid was transfected into 293 cells which had been infected with an adenovirus vector lacking the E4 region. Homologous recombination between the plasmid and the vector produced an E4-containing, replication competent vector having the chimeric NADC-1 fiber. The recombinant virus was then plaque purified on 293 cells. Preincubation of Ramos cells (which do not express a_V integrins but do express

receptors for the fiber protein of adenovirus) with recombinant NADC-1 knob blocked the transduction of these cells by the AdZ.PigSS vector, demonstrating that the vector contains a functional NADC knob. The results indicate that chimeric NADC-1 fiber can be correctly synthesized and incorporated into viable virus particles.

EXAMPLE 7

This example describes an Ad5-based adenoviral vector having a chimeric fiber trimer comprising a mutant NADC-1 knob with attenuated receptor-binding ability and containing a functional non-native ligand.

The ApaI to BamHI fragment containing the N-D mutation in pAcPig4KN N437D (Fig. 6C) is cloned into the plasmid pAcPig4KN D363E (Fig. 6B) containing the RGD-RGE mutation to create the plasmid pAcPig4KN D363E N437D (Fig. 8A) containing both mutations in the NADC-1 knob gene. Overlapping, complementary oligonucleotide primers encoding the high affinity a_v integrin binding domain, are thereafter cloned into the native AvrII site to produce the plasmid pAcPig4KN D363E N437D HAAV (Fig. 8B). The mutated NADC-1 gene fragment EcoRI to BamHI is then cloned into the plasmid pNSPig4.SS (Fig. 7B) to create the plasmid, pNS Pig4 D363E N437D HAAV SS (Fig. 8C). This plasmid is then used to create a recombinant adenovirus vector containing the mutated and a_v integrin-targeted NADC-1 knob as described above.

The ability of the double mutation in the NADC-1 knob to block binding to the native cell surface binding sites (galectin and integrin) is confirmed via competition assays. Moreover, the ability of the resultant virus to target cell-surface a_v integrin is confirmed using 293 cells, as discussed above.

EXAMPLE 8

This example describes two chimeric blocking proteins able to interfere with native adenoviral receptor binding. In particular, the blocking protein each include a domain having a substrate for the native adenovirus fiber, namely the extracellular domain of the CAR.

The extracellular domain of CAR was amplified from the CAR gene (Bergelson et al., *supra*; Tomko et al., *supra*) via PCR. The PCR product was then cloned into a baculovirus expression vector to create the plasmid pACSG2-sCAR (Fig. 9A). The soluble CAR protein (sCAR) also contained a FLAG epitope for purification and for detection by Western analysis. The DNA and amino acid sequences of this sCAR clone are set forth at SEQ ID NO:6.

Western analysis of sCAR produced in insect cells using a baculovirus clone containing sCAR revealed that the protein was secreted from the cell and that some of the protein was retained within the cell.

To assess whether the sCAR protein retains the function of the native CAR, radiolabeled adenovirus type 2 were preincubated in a solution containing various concentrations of sCAR and then exposed to 293 cells. The data demonstrated that increasing concentrations of sCAR blocked virus binding to 293 cells. This result demonstrated that the soluble sCAR protein retains the structure and function of the native extracellular domain of CAR. Moreover, these results demonstrate that preincubation with sCAR can ablate native adenoviral receptor binding via the CAR-binding ligand on the adenovirus fiber.

A second sCAR-containing chimera was produced in which DNA sequence encoding an RGD targeting motif was cloned into an SpeI site following the C-terminal end of sCAR using complementary, overlapping primers. The chimeric gene retained the FLAG epitope on the C-terminus. The resultant plasmid, SG2-sCAR-HAAV (Fig. 9B), was used to produce recombinant sCAR.RGD protein as was done for sCAR protein described above. The DNA sequence of this clone is set forth at SEQ ID NO:7.

The sCAR.RGD protein was synthesized and secreted from insect cells similarly to the sCAR protein. To assess whether the sCAR.RGD protein retains the function of the native CAR, radiolabeled adenovirus type 2 were preincubated in a solution containing various concentrations of sCAR.RGD and then exposed to Ramos cells, which do not express a_V integrins but do express receptors for the fiber protein of adenovirus. Preincubation of radiolabeled adenovirus type 2 with either sCAR or sCAR.RGD blocked virus binding to Ramos cells. This result demonstrates that the sCAR domain present in the sCAR.RGD protein is functional.

To assess whether the sCAR.RGD protein retains the function of the native RGD domain, cell adhesion studies were conducted. Both sCAR.RGD, and sCAR were immobilized onto tissue culture plastic plates, which were subsequently contacted with 293 cells (which express a_v integrin). After the cells were incubated on the coated plates, the plates were rinsed, and the number of cells remaining in contact with the plates were assayed. The results showed that cells adhered to plates coated with sCAR.RGD, while they did not adhere to plates coated with sCAR or control plates, demonstrating that the RGD motif present in the sCAR.RGD protein is functional.

EXAMPLE 9

This example demonstrates the inventive method of directing adenoviral targeting using a chimeric blocking protein having a ligand for a cell surface binding site.

An adenovirus vector carrying a lacZ reporter gene is preincubated with either the sCAR.RGD protein or the sCAR protein, described above in Example 8. The resultant complexes are then exposed to either Ramos cells (which express fiber receptor (CAR) but lack a_v integrins) or HuVEC cells (which express both CAR and a_v integrins) under

conditions suitable for viral infection. Subsequently, the cells are assayed for lacZ expression, the level of which will correlate to the degree to which the viruses infect the cells. The results will demonstrate that both sCAR and sCAR.RGD effectively block adenovirus transduction of Ramos cells whereas sCAR, but not sCAR.RGD, blocks adenovirus transduction of HuVEC cells, indicating that the Ad/sCAR.RGD complex is targeted to a_V integrins while avoiding adenoviral-mediated gene delivery to cells via CAR.

EXAMPLE 10

This example describes two chimeric blocking proteins able to form trimers interfering with native adenoviral receptor binding. In particular, the blocking proteins each include a domain having a substrate for the native adenovirus fiber, namely the extracellular domain of the CAR, and a trimerization domain, namely the sigDel trimerization domain of the Sigma-1 reovirus protein.

The sigDel trimerization domain of the Sigma-1 reovirus protein is amplified by PCR, and the resultant PCR product is cloned into the pAcSG2-sCAR plasmid (Figure 9A). The resultant plasmid, pAcSG2sCAR.sigDel (Fig. 10A) contains a gene chimera encoding the extracellular domain of CAR, a spacer region, the trimerization domain from sigma 1 protein of reovirus, and a FLAG binding domain. An SpeI restriction site following the trimerization domain allows for the convenient cloning of targeting domains, such as the high affinity RGD motif which binds a_v integrins. The DNA and amino acid sequences of this clone are set forth at SEQ ID NO:8.

PAcsCAR.sigDel was used to make baculovirus. Western analysis of boiled and unboiled cell lysates from baculovirus-infected cells showed that the unboiled chimeric sCAR.sigDel migrated as a trimer.

A second sCAR-containing chimera is produced in which DNA sequence encoding an RGD targeting motif is cloned into an SpeI site following the C-terminal end of sCAR.sigDel using complementary, overlapping primers. The resultant plasmid, pAcSG2-sCARsigDel (HAAV) (Fig. 10B), encodes a chimera having the extracellular domain of CAR, a spacer region, the trimerization domain from sigma 1 protein of reovirus, and the high affinity RGD motif which binds a_v integrins.

The pAcSG2sCAR.sigDel and pAcSG2-sCARsigDel.RGD (HAAV) plasmids were used to produce recombinant baculovirus which are used to produce the recombinant chimeric protein in insect cells by standard means. Western analysis of boiled and unboiled cell lysates from bacculovirus-infected cells demonstrated that the unboiled sCAR.sigDel protein migrated as a trimer.

To assess the ability of the trimeric sCAR.sigDel and sCARsigDel.RGD proteins to block adenoviral infection, an adenovirus vector carrying a lacZ reporter gene is

preincubated with either the sCAR.sigDel or the sCARsigDel.RGD trimer or the sCAR monomeric protein. Several concentrations are employed to generate dose-response data. The resultant complexes are then exposed to 293 cells under conditions suitable for viral infection. Subsequently, the cells are assayed for lacZ expression, the level of which will correlate to the degree to which the viruses infect the cells. The results will demonstrate that the trimeric sCAR.sigDel and sCARsigDel.RGD proteins are more potent in blocking adenovirus binding to via the sCAR protein cells than the sCAR monomers.

EXAMPLE 11

This example demonstrates the inventive method of directing adenoviral targeting using a trimeric blocking protein having a ligand for a cell surface binding site.

An adenovirus vector carrying a lacZ reporter gene is preincubated with either sCAR.sigDel, sCARsigDel.RGD, or sCAR described above. Similarly, the adenovirus can be preincubated with a blocking protein isolated, for example, by phage display. The resultant complexes are then exposed to either Ramos cells or HuVEC cells under conditions suitable for viral infection. Subsequently, the cells are assayed for lacZ expression, the level of which will correlate to the degree to which the viruses infect the cells. The results will demonstrate that, while such proteins will effectively block adenovirus transduction of Ramos cells, the trimers are more potent in blocking adenovirus binding than the sCAR monomers. Moreover, both sCAR and sCAR.sigDel, will block adenovirus transduction of HuVEC cells; however, sCARsigDel.RGD will not effectively block adenovirus transduction of HuVEC cells. Such results strongly suggests that the Ad sCARsigDel.RGD complex is targeted to a_v integrins while avoiding adenoviral-mediated gene delivery to cells via CAR.

EXAMPLE 12

This example describes the construction and evaluation of mutated fiber knobs each having reduced affinities for native substrates, particularly monoclonal antibodies raised against the native fiber knob.

Using site-directed mutagenesis, separate mutations were introduced into the full length Ad5 fiber gene in a baculoviral vector. The resultant plasmids were then used to generate recombinant baculoviral clones.

Each of the mutants, plus a native Ad5 fiber control, were used to produce protein in infected insect cells. Three days post infection, the cells were harvested and lysed. Western analysis using polyclonal antisera recognizing the Ad5 fiber revealed the presence of high amounts of fiber protein in lysates from cells infected with each of the vectors. In cells infected with five of the mutant clones (see table 1) (as well as the native fiber gene), the signal was predominantly in the soluble portion of the lysates, indicating

that the protein encoded by each mutant was correctly folded. The sequences of the wild-type Ad5 fiber is set forth a SEQ ID NO:9. The amino acids of SEQ ID NO:9 changed by each of these mutations is indicated in Table 1.

Table 1

Mutations	Monoclonal Antibodies			
	2C9	**4B8**	**3D9**	**2E5**
CD Loop (449 SGTVQ-GSGSG)	-	-	+	+
IJ Loop (559 GSHN-GSGS)	-	-	+	+
FG Loop (507 SHGKTA-GSGSGS)	-	-	+	+
T533S/T353S (535 TIT-SIS)	+	-	+	+
K506R (506 K-R)	+	+	-	+
C-Term Addition	+	+	+	+
Native Ad5 Fiber	+	+	+	+
Boiled Ad5 Fiber	-	-	-	-

Using Western slot-blot analysis, each of the five soluble mutant fiber proteins, the native Ad5 fiber, and a denatured Ad5 fiber were screened against a panel of four monoclonal antibodies raised against the fiber knob. The signals were detected by chemiluminescence and the strength of signals of each band compared. The results of this assay are set forth in Table 1.

That none of the antibodies recognizes the denatured fiber demonstrates that each binds only correctly folded, trimeric fibers. Furthermore, that none of the mutants exhibited reduced affinity for the 2E5 antibody confirmed that each of the mutant fibers was, indeed, trimeric.

The K506R mutation significantly reduced the affinity of the resultant fiber for the 3D9 antibody without affecting the affinity for any of the other antibodies. The location of this mutation within the fiber knob is indicated in Figs. 15A-15C.

Mutations in the CD, IJ, or FG loops, in which 4-6 amino acids were replaced by altering serines and glycines, significantly reduced the affinity of the resultant mutant trimers for the 2C9 antibody. Moreover, the double mutant T533S/T535S also reduced the affinity of the mutant knob for the 4B8 antibody. The location of each of these mutations within the fiber knob are indicated in Figs. 15D-15F.

These results indicate that the trimeric fiber knobs having reduced affinity for native substrates can be generated. A similar screening protocol can be used to identify mutants having reduced affinity for cellular receptors. For example, a soluble form of sCAR having a FLAG epitope (or other tag), such as described above, can be used as a probe in place of the monoclonal antibodies described above. The blots are then screened

with anti-FLAG monoclonal antibodies to detect mutations interfering with fiber-CAR binding.

EXAMPLE 13

This example describes the construction of a recombinant adenovirus containing a short-shafted fiber (e.g., 8 shaft repeats) and a mutant fiber(5) knob having reduced affinity for its native receptor (i.e., CAR). Such a fiber permits targeting via a ligand expressed in the penton base.

Using standard recombination techniques, a deletion is introduced into the sequence encoding the fiber shaft. For example, a portion of the mutant fiber knob from the 22^{d} shaft repeat until the end of the coding sequence and containing the K506R mutation (see Example 12) is amplified by PCR from SEQ ID NO:8. The resultant product is used to create the pAS T5S7F5K(R506K) plasmid (Fig. 16). The plasmid, thus, contains a gene encoding a short-shafted fiber with reduced affinity for a native substrate (the 3D9 antibody). An adenovirus having such a short-shafted fiber will be able to bind to cells via the RGD ligand on the penton base. Of course, a similar strategy can be used to create adenoviral vectors having short-shafted fibers with reduced affinity for the CAR.

EXAMPLE 14

This example demonstrates the construction of adenovirus vectors having specific non-native ligands that can be used to purify the vector via affinity chromatography.

The base vector pNSF5F2K (Figure 8A) contains a gene which encodes a chimeric fiber having the shaft of the Ad5 fiber and the knob of the Ad2 fiber protein. The Ad2 fiber gene contains an SpeI restriction site in the region of the knob which encodes the flexible, exposed HI loop of the fiber knob. This SpeI restriction site was used to insert sequences which encode the FLAG peptide SEQ ID NO:16 or a DNA/heparin-binding ligand (SEQ ID NO:15).

The base vector pBSSpGS (Figure 11A) encodes a C-terminal 12 amino acid extension (SEQ ID NO:14). The codons encoding the TS also are a unique SpeI site that was used to insert sequences which encode the FLAG peptide (SEQ ID NO:16) or the DNA/heparin-binding polypeptide (SEQ ID NO:15) as described below.

Transfer plasmids (pBSS pGS (RKKK)2 (Figure 11B) and pNSF5F2K(RKKK)2 (Figure 11C)) for introducing the DNA/heparin-binding ligand into the adenoviral genome were created using overlapping oligonucleotides. Sense and antisense oligonucleotides were mixed in equimolar ratios and cloned into the SpeI site of pBSS pGS (Fig. 11A) or pNS F5F2K (Fig. 8A) to create the transfer plasmids. Sequencing in

both directions across the region of the inserts verified that the clones contained the appropriate sequence.

Similarly, transfer plasmids pBSSpGS (FLAG) (Figure 11D) and pNSF5F2K(FLAG) (Figure 11E) for introducing the FLAG ligand (SEQ ID NO:16) into the adenoviral genome were created. Sequencing in both directions across the region of the inserts verified that the clones contained the appropriate sequence.

The plasmid DNA from the four transfer vectors were linearized with SalI, purified and transfected using calcium phosphate into 293 cells which had been preincubated for 1 h with the E1, E3, E4-deleted adenovirus AdCMVZ.11A (GenVec, Inc., Rockville, MD) a multiplicity of 1 ffu per cell. Recombination of the E4+ pNS plasmid with the E4-deleted vector resulted in the rescue of an E1-, E3-, E4+ vector capable of replication in 293 cells. The resultant vectors, AdZ.F2K(RKKK)2, AdZ.F2K(FLAG), AdZ.F(RKKK)2 and AdZ.F(FLAG), were isolated in two successive rounds of plaquing on 293 cells.

Each vector was verified to contain the correct insert by sequencing PCR products derived from virus DNA template using primers spanning the region of the insert DNA. Restriction analysis of Ad DNA from each of the viruses showed that the viruses were pure and contained the BamHI restriction site unique to the correctly constructed virus.

EXAMPLE 15

This example demonstrates that an adenoviral vector having a non-native ligand can bind a support conjugated to a substrate for that ligand.

The vector AdZ.PK was constructed similarly to the vectors described above; the virus has a fiber protein containing polylysines. AdZ.PK was assayed to determine whether the virus could bind a support having a substrate for polylisine, heparin. 50 ml of heparin-agarose beads (SIGMA) were added to 1.0 ml of phosphate buffers containing 150, 300, 500 and 1000 mM NaCl, respectively. 6600 cpm of either AdZ or AdZ.PK were then added to the saline buffers containing the heparin-agarose beads and rocked for 60 min. The beads were then washed three times with a buffer of equal salinity to the incubation buffer (150, 300, 500, and 1000 mM NaCl, respectively). The bead-associated cpm were then measured and showed the preferential binding of AdZ.PK over AdZ at 150, 300, and 500 mM NaCl. However, at 1000 mM NaCl the binding of AdZ.PK to the beads was much lower and approximately equal to the background binding observed for AdZ.

These results demonstrate that the AdZ.PK vector binds a heparin-linked support material and that binding is ablated by high salt concentration. Therefore, such a support can be used to purify the modified vector by first binding the virus to the support at low salt conditions and then eluting the vector at high salt conditions.

EXAMPLE 16

This example demonstrates that an adenoviral vector having a non-native ligand can be purified on a column comprising substrate for that ligand.

20 175 cm2 tissue culture flasks containing 293 packaging cell lines are infected at an m.o.i. of 5 with one of the three vectors: AdZ.PK, AdZ.F2K(RKKK)2 or AdZ.F(RKKK)2 described above. The cells are then incubated for 2 days, after which any remaining adherent cells are then dislodged from the plastic. The removed cells are centrifuged at 3,000 g to form a pellet, the culture medium removed, and the pellet gently washed 2 times with PBS. The cells are then resuspended in a total volume of 5 ml PBS containing 10 mM $MgCl_2$.

The resuspended cells are then freeze-thawed 3 times to release the virus, and the cell debris is then centrifuged at 15,000 g for 15 min. The supernatant is passed over a 3 ml column containing heparin-linked agarose beads. The column is then washed with 30 ml of PBS followed by elution of the virus from the column by a salt step gradient. To elute the virus, 3 ml volumes of buffers containing successively larger concentrations of NaCl (in 100 mM steps) are successively passed over the column, and 1 ml elution volumes are collected (3 ml 200 mM NaCl; 3 ml 300 mM NaCl; 3 ml 400 mM NaCl; up to 2000 mM NaCl).

The fractions, including the runthrough and wash fractions, are then evaluated for adenovirus coat proteins by Western blot, for active virus particles by lacZ transduction levels of A549 cells or by plaque assay, and for overall purity by analytical high performance liquid chromatography (HPLC) as previously described (Shabram, et al, 1997, *Hum. Gene Ther. 8*, 453-46; Huyghe, et al, 1995, *Hum. Gene Ther.*, *6*: 1403-1416; Shabram et al, WO 96/27677). The overall purity of the fractions determined to contain peak adenovirus concentrations is evaluated by running the fractions on HPLC and comparing the profile to a pre-column fraction and a highly purified adenovirus preparation (prepared by 3 successive rounds of purification on CsCl gradients).

EXAMPLE 17

This example describes the production of a pseudo-receptor for constructing a cell line able to replicate adenoviruses lacking native cell-binding function (but targeted for the pseudo-receptor). Specifically, the exemplary pseudo-receptor includes a binding domain from a single-chain antibody (ScFv).

First a vector expressing the ScFv from pHOOK3 (Figure 12A) (Invitrogen), which encodes a ScFv synthesized with a murine Ig signal peptide. The ScFv has an N-terminal HA epitope tag, and its C-terminus is linked to a pair of myc epitopes followed by the PDGF receptor transmembrane anchor. An expression cassette including this

construct was cloned into plasmid pRC/CMVp-Puro (Fig. 12B) to create the pScHAHK plasmid (Fig. 12C). This plasmid has cloning sites for inserting genes after the CMV promoter and unique AgeI and XbaI sites for the addition of cytoplasmic sequences at the C-terminus of the gene.

To demonstrate cell-surface expression of the ScFv pseudo-receptor, either the pNSE4GLP plasmid alone (Figure 12D), which carries a green fluorescent protein gene for detection of tranfectants, or in combination with pScHAHK, were transfected into 293 cells. One day post transfection, the pScHAHK-exhibited surface immunofluorescence using an antibody directed to the HA epitope, demonstrating proper surface expression of the pseudo-receptor.

To demonstrate that the expressed pseudo-receptor is functional, transfected cells were exposed to magnetic CAPTURE-TEC beads conjugated with antigens recognized by the ScFv. Following incubation, the beads were collected in the bottom of a tube using a magnet, washed, and transferred to a culture dish. The culture dishes were then viewed under a fluorescence microscope to identify GFP-expressing cells. No staining was observed from cells transfected only with pNSE4GLP alone, indicating that these cells did not bind the beads. However, cells transfected with pNSE4GLP and pScHAHK were observed in the wells. This result demonstrates that the doubly transfected cells bound to the beads.

EXAMPLE 18

This example describes the production of a pseudo-receptor for constructing a cell line able to replicate adenoviruses lacking native cell-binding function (but targeted for the pseudo-receptor). Specifically, the exemplary pseudo-receptor includes a binding domain from a single-chain antibody recognizing HA.

Anti-HA ScFv was constructed as an N-Term-VL-VH fusion protein. RT-PCR was performed on RNA obtained from hybridomas producing HA antibodies using primers specific for κ- or γ2β- and C-terminus of the VL and VH genes (see Gilliland et al., *Tissue Antigens, 47*, 1-20 (1996)). After sequencing the resulting PCR products, specific oligonucleotides were designed to amplify the VL-VH fusion in a second round of PCR. The final PCR product was cloned to create the pCANTAB5E(HA) plasmid (Fig. 17A) for production of anti HA ScFv in *E. coli*. The expressed protein has a C-terminal E peptide for detection of binding to HA-tagged penton base via Western analysis of ELISA assay. Upon transformation of bacterial cells with the pCANTAB5E(HA) plasmid, Western analysis using an antibody recognizing the E peptide revealed a protein of the expected size.

To determine whether the anti-HA ScFv was functional, it was used in protein A immunoprecipitation assays using adenoviral coat proteins (recombinant penton base)

containing the HA epitope. The anti-HA ScFv was able to precipitate HA-containing penton base proteins. These results indicate the successful construction of the extracellular portion of a pseudo-receptor for binding an adenovirus having a non-native ligand (i.e., HA).

To create an entire anti-HA pseudo-receptor, the anti-HA ScFv was cloned into the pSCHAHK plasmid in which the HA had been removed to create the pScFGHA plasmid (Fig. 17B). This plasmid will produce an anti-HA pseudo-receptor able to bind recombinant adenoviruses having the HA epitope, similar to adenoviruses described above having FLAG epitopes.

EXAMPLE 19

This example describes the creation of a fiber-expressing cell line for the production of targeted adenovirus particles. The complementing cell line produces a fiber protein with or without additional complementary genes from the adenovirus genome.

The entire adenovirus type 2 fiber gene was amplified from adenovirus type 2 DNA by PCR. The resultant product was cloned into the pCR2.1-TOPO plasmid (Invitrogen) to make the plasmid pCR2.1-TOPO+fiber (Fig. 13A). The fiber2 gene was then excised from the pCR2.1-TOPO+fiber plasmid with the restriction enzymes BamHI and EagI, and it was then subcloned into the plasmid, pKSII (Stratagene), to construct the plasmid pKSII Fiber (Fig. 13B). The fiber2 gene was then excised from the pKSII Fiber plasmid using the restriction enzymes KpnI and EagI, and it was then cloned into the plasmid, pSMTZeo-DBP (Fig. 13C). The resultant plasmid, pSMTZeo-Fiber (Fig. 13D), encoded the entire fiber2 gene under control of the metallothionine promoter. This construct also placed an efficient mRNA splice site before the fiber gene to enhance fiber protein synthesis following induction. The pSMTZeo-Fiber plasmid also contains a Zeo resistance marker to allow selection of cell lines on the antibiotic zeocin.

To produce the cell line, the pSMTZeo-Fiber plasmid is transfected into 293 cells (or some other cell line) with or without additional adenovirus complementing functions. Individual zeocin-resistant cell colonies are then amplified by standard means and tested for fiber2 production (e.g., by Western analysis using an anti-fiber2 antibody) before and after induction with zinc, which activates the metallothionine promoter. Selected fiber-expressing clones are then tested for the ability to plaque and/or complement the growth of adenoviruses containing mutated fibers. Clones that adequately complement mutated fibers are suitable for amplifying and growing adenovirus particles having genomes encoding mutant fiber genes.

All references cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

While this invention has been described with an emphasis on preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments can be used and that it is intended that the invention can be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

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(ii) TITLE OF INVENTION: ALTERNATIVELY TARGETED ADENOVIRUS

(iii) NUMBER OF SEQUENCES: 18

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

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(B) FILING DATE: 16-JAN-1998

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 960 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..957

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATG AAG CGC GCA AGA CCG TCT GAA GAT ACC TTC AAC CCC GTG TAT CCA        48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
 1               5                  10                  15

TAT GAC ACG GAA ACC GGT CCT CCA ACT GTG CCT TTT CTT ACT CCT CCC        96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
            20                  25                  30

TTT GTA TCC CCC AAT GGG TTT CAA GAG AGT CCC CCC GGG GTA CTC TCT       144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
        35                  40                  45

TTG CGC CTA TCC GAA CCT CTA GTT ACC TCC AAT GGC ATG CTT GCG CTC       192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
    50                  55                  60

AAA ATG GGC AAC GGC CTC TCT CTG GAC GAG GCC GGC AAC CTT ACC TCC       240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65                  70                  75                  80

CAA AAT GTA ACC ACT GTG AGC CCA CCT CTC AAA AAA ACC AAG TCA AAC       288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
                85                  90                  95

ATA AAC CTG GAA ATA TCT GCA CCC CTC ACA GTT ACC TCA GAA GCC CTA       336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
            100                 105                 110

ACT GTG GCT GCC GCC GCA CCT CTA ATG GTC GCG GGC AAC ACA CTC ACC       384
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
        115                 120                 125

ATG CAA TCA CAG GCC CCG CTA ACC GTG CAC GAC TCC AAA CTT AGC ATT       432
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
    130                 135                 140
```

```
GCC ACC CAA GGA CCC CTC ACA GTG TCA GAA GGA AAG CTA GCA TCA AGG      480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Ser Arg
145                 150                 155                 160

GTC TCG GCG CTC GAG AAG ACG TCT CAA ATA CAC TCT GAT ACT ATC CTC      528
Val Ser Ala Leu Glu Lys Thr Ser Gln Ile His Ser Asp Thr Ile Leu
                165                 170                 175

CGG ATC ACC CAG GGA CTC GAT GAT GCA AAC AAA CGA ATC ATC GCT CTT      576
Arg Ile Thr Gln Gly Leu Asp Asp Ala Asn Lys Arg Ile Ile Ala Leu
            180                 185                 190

GAG CAA AGT CGG GAT GAC TTG GTT GCA TCA GTC AGT GAT GCT CAA CTT      624
Glu Gln Ser Arg Asp Asp Leu Val Ala Ser Val Ser Asp Ala Gln Leu
        195                 200                 205

GCA ATC TCC AGA TTG GAA AGC TCT ATC GGA GCC CTC CAA ACA GTT GTC      672
Ala Ile Ser Arg Leu Glu Ser Ser Ile Gly Ala Leu Gln Thr Val Val
    210                 215                 220

AAT GGA CTT GAT TCG AGT GTT ACC CAG TTG GGT GCT CGA GTG GGA CAA      720
Asn Gly Leu Asp Ser Ser Val Thr Gln Leu Gly Ala Arg Val Gly Gln
225                 230                 235                 240

CTT GAG ACA GGA CTT GCA GAC GTA CGC GTT GAT CAC GAC AAT CTC GTT      768
Leu Glu Thr Gly Leu Ala Asp Val Arg Val Asp His Asp Asn Leu Val
                245                 250                 255

GCG AGA GTG GAT ACT GCA GAA CGT AAC ATT GGA TCA TTG ACC ACT GAG      816
Ala Arg Val Asp Thr Ala Glu Arg Asn Ile Gly Ser Leu Thr Thr Glu
            260                 265                 270

CTA TCA ACT CTG ACG TTA CGA GTA ACA TCC ATA CAA GCG GAT TTC GAA      864
Leu Ser Thr Leu Thr Leu Arg Val Thr Ser Ile Gln Ala Asp Phe Glu
        275                 280                 285

TCT AGG GGA TCC GGC GGC ACT AGT GGC GGC GAC TAC AAG GAC GAC GAC      912
Ser Arg Gly Ser Gly Gly Thr Ser Gly Gly Asp Tyr Lys Asp Asp Asp
    290                 295                 300

GAC AAG GGC CCT AGG GGC GCC CGC CGC GCC TCC CTT GGC TCT AGA TAA      960
Asp Lys Gly Pro Arg Gly Ala Arg Arg Ala Ser Leu Gly Ser Arg
305                 310                 315
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 633 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..630

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
ATG AAG CGC GCA AGA CCG TCT GAA GAT ACC TTC AAC CCC GTG TAT CCA       48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
                325                 330                 335

TAT GAC ACG GAA ACC GGT CCT CCA ACT GTG CCT TTT CTT ACT CCT CCC       96
```

```
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
            340                 345                 350

TTT GTA TCC CCC AAT GGG TTT CAA GAG AGT CCC CCC GGG GGA GGG CTA      144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Gly Gly Leu
        355                 360                 365

GCA TCA AGG GTC TCG GCG CTC GAG AAG ACG TCT CAA ATA CAC TCT GAT      192
Ala Ser Arg Val Ser Ala Leu Glu Lys Thr Ser Gln Ile His Ser Asp
    370                 375                 380

ACT ATC CTC CGG ATC ACC CAG GGA CTC GAT GAT GCA AAC AAA CGA ATC      240
Thr Ile Leu Arg Ile Thr Gln Gly Leu Asp Asp Ala Asn Lys Arg Ile
385                 390                 395                 400

ATC GCT CTT GAG CAA AGT CGG GAT GAC TTG GTT GCA TCA GTC AGT GAT      288
Ile Ala Leu Glu Gln Ser Arg Asp Asp Leu Val Ala Ser Val Ser Asp
                405                 410                 415

GCT CAA CTT GCA ATC TCC AGA TTG GAA AGC TCT ATC GGA GCC CTC CAA      336
Ala Gln Leu Ala Ile Ser Arg Leu Glu Ser Ser Ile Gly Ala Leu Gln
            420                 425                 430

ACA GTT GTC AAT GGA CTT GAT TCG AGT GTT ACC CAG TTG GGT GCT CGA      384
Thr Val Val Asn Gly Leu Asp Ser Ser Val Thr Gln Leu Gly Ala Arg
        435                 440                 445

GTG GGA CAA CTT GAG ACA GGA CTT GCA GAC GTA CGC GTT GAT CAC GAC      432
Val Gly Gln Leu Glu Thr Gly Leu Ala Asp Val Arg Val Asp His Asp
    450                 455                 460

AAT CTC GTT GCG AGA GTG GAT ACT GCA GAA CGT AAC ATT GGA TCA TTG      480
Asn Leu Val Ala Arg Val Asp Thr Ala Glu Arg Asn Ile Gly Ser Leu
465                 470                 475                 480

ACC ACT GAG CTA TCA ACT CTG ACG TTA CGA GTA ACA TCC ATA CAA GCG      528
Thr Thr Glu Leu Ser Thr Leu Thr Leu Arg Val Thr Ser Ile Gln Ala
                485                 490                 495

GAT TTC GAA TCT AGG GGA TCC GGC GGC ACT AGT GGC GGC GAC TAC AAG      576
Asp Phe Glu Ser Arg Gly Ser Gly Gly Thr Ser Gly Gly Asp Tyr Lys
            500                 505                 510

GAC GAC GAC GAC AAG GGC CCT AGG GGC GCC CGC CGC GCC TCC CTT GGC      624
Asp Asp Asp Asp Lys Gly Pro Arg Gly Ala Arg Arg Ala Ser Leu Gly
        515                 520                 525

TCT AGA TAA                                                         633
Ser Arg
    530
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1704 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..1701

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG AAG CGC GCA AGA CCG TCT GAA GAT ACC TTC AAC CCC GTG TAT CCA        48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
            215                 220                 225

TAT GAC ACG GAA ACC GGT CCT CCA ACT GTG CCT TTT CTT ACT CCT CCC        96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
        230                 235                 240

TTT GTA TCC CCC AAT GGG TTT CAA GAG AGT CCC CCC GGG GTA CTC TCT       144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
    245                 250                 255

TTG CGC CTA TCC GAA CCT CTA GTT ACC TCC AAT GGC ATG CTT GCG CTC       192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
260                 265                 270                 275

AAA ATG GGC AAC GGC CTC TCT CTG GAC GAG GCC GGC AAC CTT ACC TCC       240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
                280                 285                 290

CAA AAT GTA ACC ACT GTG AGC CCA CCT CTC AAA AAA ACC AAG TCA AAC       288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
            295                 300                 305

ATA AAC CTG GAA ATA TCT GCA CCC CTC ACA GTT ACC TCA GAA GCC CTA       336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
        310                 315                 320

ACT GTG GCT GCC GCC GCA CCT CTA ATG GTC GCG GGC AAC ACA CTC ACC       384
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
    325                 330                 335

ATG CAA TCA CAG GCC CCG CTA ACC GTG CAC GAC TCC AAA CTT AGC ATT       432
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
340                 345                 350                 355

GCC ACC CAA GGA CCC CTC ACA GTG TCA GAA GGA AAG CTA GCA TCA AGG       480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Ser Arg
                360                 365                 370

GTC TCG GCG CTC GAG AAG ACG TCT CAA ATA CAC TCT GAT ACT ATC CTC       528
Val Ser Ala Leu Glu Lys Thr Ser Gln Ile His Ser Asp Thr Ile Leu
            375                 380                 385

CGG ATC ACC CAG GGA CTC GAT GAT GCA AAC AAA CGA ATC ATC GCT CTT       576
Arg Ile Thr Gln Gly Leu Asp Asp Ala Asn Lys Arg Ile Ile Ala Leu
        390                 395                 400

GAG CAA AGT CGG GAT GAC TTG GTT GCA TCA GTC AGT GAT GCT CAA CTT       624
Glu Gln Ser Arg Asp Asp Leu Val Ala Ser Val Ser Asp Ala Gln Leu
    405                 410                 415

GCA ATC TCC AGA TTG GAA AGC TCT ATC GGA GCC CTC CAA ACA GTT GTC       672
Ala Ile Ser Arg Leu Glu Ser Ser Ile Gly Ala Leu Gln Thr Val Val
420                 425                 430                 435

AAT GGA CTT GAT TCG AGT GTT ACC CAG TTG GGT GCT CGA GTG GGA CAA       720
Asn Gly Leu Asp Ser Ser Val Thr Gln Leu Gly Ala Arg Val Gly Gln
                440                 445                 450

CTT GAG ACA GGA CTT GCA GAC GTA CGC GTT GAT CAC GAC AAT CTC GTT       768
Leu Glu Thr Gly Leu Ala Asp Val Arg Val Asp His Asp Asn Leu Val
            455                 460                 465

GCG AGA GTG GAT ACT GCA GAA CGT AAC ATT GGA TCA TTG ACC ACT GAG       816
```

```
Ala Arg Val Asp Thr Ala Glu Arg Asn Ile Gly Ser Leu Thr Thr Glu
        470                 475                 480

CTA TCA ACT CTG ACG TTA CGA GTA ACA TCC ATA CAA GCG GAT TTC GAA      864
Leu Ser Thr Leu Thr Leu Arg Val Thr Ser Ile Gln Ala Asp Phe Glu
    485                 490                 495

TCT AGG GGA TCC GGC GGC ACT AGA GGA GGT GGA ATG AGC AAG GGC GAG      912
Ser Arg Gly Ser Gly Gly Thr Arg Gly Gly Gly Met Ser Lys Gly Glu
500                 505                 510                 515

GAA CTG TTC ACT GGC GTG GTC CCA ATT CTC GTG GAA CTG GAT GGC GAT      960
Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp
                520                 525                 530

GTG AAT GGG CAC AAA TTT TCT GTC AGC GGA GAG GGT GAA GGT GAT GCC      1008
Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala
            535                 540                 545

ACA TAC GGA AAG CTC ACC CTG AAA TTC ATC TGC ACC ACT GGA AAG CTC      1056
Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu
        550                 555                 560

CCT GTG CCA TGG CCA ACA CTG GTC ACT ACC TTC ACC TAT GGC GTG CAG      1104
Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Thr Tyr Gly Val Gln
    565                 570                 575

TGC TTT TCC AGA TAC CCA GAC CAT ATG AAG CAG CAT GAC TTT TTC AAG      1152
Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys
580                 585                 590                 595

AGC GCC ATG CCC GAG GGC TAT GTG CAG GAG AGA ACC ATC TTT TTC AAA      1200
Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys
                600                 605                 610

GAT GAC GGG AAC TAC AAG ACC CGC GCT GAA GTC AAG TTC GAA GGT GAC      1248
Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp
            615                 620                 625

ACC CTG GTG AAT AGA ATC GAG TTG AAG GGC ATT GAC TTT AAG GAA GAT      1296
Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp
        630                 635                 640

GGA AAC ATT CTC GGC CAC AAG CTG GAA TAC AAC TAT AAC TCC CAC AAT      1344
Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn
    645                 650                 655

GTG TAC ATC ATG GCC GAC AAG CAA AAG AAT GGC ATC AAG GTC AAC TTC      1392
Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe
660                 665                 670                 675

AAG ATC AGA CAC AAC ATT GAG GAT GGA TCC GTG CAG CTG GCC GAC CAT      1440
Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His
                680                 685                 690

TAT CAA CAG AAC ACT CCA ATC GGC GAC GGC CCT GTG CTC CTC CCA GAC      1488
Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp
            695                 700                 705

AAC CAT TAC CTG TCC ACC CAG TCT GCC CTG TCT AAA GAT CCC AAC GAA      1536
Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu
        710                 715                 720

AAG AGA GAC CAC ATG GTC CTG CTG GAG TTT GTG ACC GCT GCT GGG ATC      1584
Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile
    725                 730                 735
```

```
ACA CAT GGC ATG GAC GAG CTG TAC AAG GGT GGA GGT AGA TCT ACT AGT      1632
Thr His Gly Met Asp Glu Leu Tyr Lys Gly Gly Gly Arg Ser Thr Ser
740                 745                 750                 755

GGC GGC GAC TAC AAG GAC GAC GAC GAC AAG GGC CCT AGG GGC GCC CGC      1680
Gly Gly Asp Tyr Lys Asp Asp Asp Asp Lys Gly Pro Arg Gly Ala Arg
                760                 765                 770

CGC GCC TCC CTT GGC TCT AGA TAA                                      1704
Arg Ala Ser Leu Gly Ser Arg
            775
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1830 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..1827

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
ATG AGA GGA TCT CAC CAT CAC CAT CAC CAT GGC GAA GAT GGA GCT TTG       48
Met Arg Gly Ser His His His His His His Gly Glu Asp Gly Ala Leu
    570                 575                 580

TCC CTG ACA AAA ACC TTA GTC TAT CCC ACC CTG TGG ACG GGG CCT GCT       96
Ser Leu Thr Lys Thr Leu Val Tyr Pro Thr Leu Trp Thr Gly Pro Ala
585                 590                 595                 600

CCC GAG GCC AAC GTC ACC TTC TCG GGG GAG AAT TCC CCA TCT GGC ATT      144
Pro Glu Ala Asn Val Thr Phe Ser Gly Glu Asn Ser Pro Ser Gly Ile
                605                 610                 615

CTC AGA CTG TGT CTC AGC AGA ACC GGG GGC ACG GTC ATT GGC ACC CTG      192
Leu Arg Leu Cys Leu Ser Arg Thr Gly Gly Thr Val Ile Gly Thr Leu
            620                 625                 630

TCT GTA CAA GGT AGC CTC ACG AAC CCC AGT ACC GGT CAG ACC CTG GGC      240
Ser Val Gln Gly Ser Leu Thr Asn Pro Ser Thr Gly Gln Thr Leu Gly
        635                 640                 645

ATG AAC CTT TAC TTT GAC GCA GAC GGC AAT GTG CTG TCT GAG AGC AAC      288
Met Asn Leu Tyr Phe Asp Ala Asp Gly Asn Val Leu Ser Glu Ser Asn
    650                 655                 660

CTC GTC CGA GGG TCC TGG GGA ATG AAA GAC CAA GAT ACC CTG GTG ACT      336
Leu Val Arg Gly Ser Trp Gly Met Lys Asp Gln Asp Thr Leu Val Thr
665                 670                 675                 680

CCC ATT GCC AAT GGG CAG TAC CTG ATG CCC AAC CTC ACT GCA TAC CCT      384
Pro Ile Ala Asn Gly Gln Tyr Leu Met Pro Asn Leu Thr Ala Tyr Pro
                685                 690                 695

CGC CTC ATA CAG ACC CTA ACT TCC AGC TAC ATT TAC ACA CAA GCG CAC      432
Arg Leu Ile Gln Thr Leu Thr Ser Ser Tyr Ile Tyr Thr Gln Ala His
            700                 705                 710

CTT GAC CAC AAT AAC AGT GTG GTG GAC ATC AAG ATA GGG CTC AAC ACA      480
```

```
Leu Asp His Asn Asn Ser Val Val Asp Ile Lys Ile Gly Leu Asn Thr
        715                 720                 725

GAC CTG AGG CCC ACT GCG GCC TAC GGC CTA AGC TTT ACC ATG ACC TTC      528
Asp Leu Arg Pro Thr Ala Ala Tyr Gly Leu Ser Phe Thr Met Thr Phe
    730                 735                 740

ACT AAC TCT CCC CCC ACC TCA TTT GGT ACC GAC CTG GTG CAA TTT GGC      576
Thr Asn Ser Pro Pro Thr Ser Phe Gly Thr Asp Leu Val Gln Phe Gly
745                 750                 755                 760

TAC CTG GGT CAG GAT AGC TCC CCC TCC TTC CTG AGA GAA CTT CCC CTT      624
Tyr Leu Gly Gln Asp Ser Ser Pro Ser Phe Leu Arg Glu Leu Pro Leu
                765                 770                 775

GCA TCC GAG GCG GGC TAC TTT GGC AAA CTG GCA GCT GCC TCT GAG GAA      672
Ala Ser Glu Ala Gly Tyr Phe Gly Lys Leu Ala Ala Ala Ser Glu Glu
            780                 785                 790

ATG CCA GCC CCT CCT GAG GCC CAG ACG CAG GAC CAA GCA GCT GAG GAG      720
Met Pro Ala Pro Pro Glu Ala Gln Thr Gln Asp Gln Ala Ala Glu Glu
        795                 800                 805

CCC CCG GCT CCT GCT GAG GCT GAG GCC CCC GCT CCT GCT GAG GCT GAG      768
Pro Pro Ala Pro Ala Glu Ala Glu Ala Pro Ala Pro Ala Glu Ala Glu
    810                 815                 820

GCT GAG GCT GAA CCG CCC CGA AAA CCC CCT AGG GGT GAC CTG GCC GCC      816
Ala Glu Ala Glu Pro Pro Arg Lys Pro Pro Arg Gly Asp Leu Ala Ala
825                 830                 835                 840

CTA TAC AAT AGG GTC CAC AGC GAC ACC CGC GCA GAG GAC ACA CCA ACC      864
Leu Tyr Asn Arg Val His Ser Asp Thr Arg Ala Glu Asp Thr Pro Thr
                845                 850                 855

AGC CCC GAG TTG GTC ACA ACC TTG CCA GAC CCC TTT GTC CTC CCC CTA      912
Ser Pro Glu Leu Val Thr Thr Leu Pro Asp Pro Phe Val Leu Pro Leu
            860                 865                 870

CCC GAC GGA GTC CCA ACC GGT GCG AGC ATT GTG TTG GAA GGT ACC CTC      960
Pro Asp Gly Val Pro Thr Gly Ala Ser Ile Val Leu Glu Gly Thr Leu
        875                 880                 885

ACA CCC TCC GCT GTG TTT TTT ACC CTG GAT CTG GTG ACC GGG CCC GCC     1008
Thr Pro Ser Ala Val Phe Phe Thr Leu Asp Leu Val Thr Gly Pro Ala
    890                 895                 900

AGT CTG GCG CTG CAC TTT AAC GTG CGC CTC CCA CTG GAA GGC GAA AAG     1056
Ser Leu Ala Leu His Phe Asn Val Arg Leu Pro Leu Glu Gly Glu Lys
905                 910                 915                 920

CAC ATT GTG TGC AAC TCC AGA GAG GGT AGC AGC AAC TGG GGC GAA GAA     1104
His Ile Val Cys Asn Ser Arg Glu Gly Ser Ser Asn Trp Gly Glu Glu
                925                 930                 935

GTA AGA CCG CAG GAG TTC CCC TTT GAA AGG GAA AAG CCA TTC GTC CTG     1152
Val Arg Pro Gln Glu Phe Pro Phe Glu Arg Glu Lys Pro Phe Val Leu
            940                 945                 950

GTC ATT GTC ATC CAA AGT GAC ACA TAC CAG ATC ACT GTG AAC GGG AAG     1200
Val Ile Val Ile Gln Ser Asp Thr Tyr Gln Ile Thr Val Asn Gly Lys
        955                 960                 965

CCT CTG GTG GAT TTT CCA CAG AGA CTA CAG GGC ATT ACC CGT GCC TCC     1248
Pro Leu Val Asp Phe Pro Gln Arg Leu Gln Gly Ile Thr Arg Ala Ser
    970                 975                 980
```

```
CTA TCC GGA GAC CTT GTG TTT ACC CGG TTG ACA ATG TAC CCA CCC GGA      1296
Leu Ser Gly Asp Leu Val Phe Thr Arg Leu Thr Met Tyr Pro Pro Gly
985                 990                 995                 1000

GAC CCC CGT CCC ACA ACC TTG TTA CCA CCC CCC GCA GCT CCC CTG GAC      1344
Asp Pro Arg Pro Thr Thr Leu Leu Pro Pro Pro Ala Ala Pro Leu Asp
                1005                1010                1015

GTA ATC CCA GAT GCC TAT GTG CTC AAT CTG CCC ACC GGA CTG ACG CCT      1392
Val Ile Pro Asp Ala Tyr Val Leu Asn Leu Pro Thr Gly Leu Thr Pro
          1020                1025                1030

AGA ACA CTC CTC ACC GTC ACG GGA ACC CCC ACG CCC CTC GCC GAA TTT      1440
Arg Thr Leu Leu Thr Val Thr Gly Thr Pro Thr Pro Leu Ala Glu Phe
      1035                1040                1045

TTT ATT GTG AAT CTG GTC TAC GAT TTA CAC TAT GAT TCC AAA AAT GTG      1488
Phe Ile Val Asn Leu Val Tyr Asp Leu His Tyr Asp Ser Lys Asn Val
  1050                1055                1060

GCC CTC CAC TTT AAT GTC GGC TTC ACC TCT GAC AGC AAA GGC CAC ATC      1536
Ala Leu His Phe Asn Val Gly Phe Thr Ser Asp Ser Lys Gly His Ile
1065                1070                1075                1080

GCC TGC AAT GCC AGA ATG AAT GGC ACA TGG GGA AGT GAA ATC ACA GTG      1584
Ala Cys Asn Ala Arg Met Asn Gly Thr Trp Gly Ser Glu Ile Thr Val
                1085                1090                1095

TCT GAT TTC CCC TTT CAA AGG GGA AAA CCC TTC ACT CTG CAG ATT CTC      1632
Ser Asp Phe Pro Phe Gln Arg Gly Lys Pro Phe Thr Leu Gln Ile Leu
          1100                1105                1110

ACC AGA GAG GCA GAC TTC CAA GTC CTC GTA GAT AAA CAA CCT TTA ACC      1680
Thr Arg Glu Ala Asp Phe Gln Val Leu Val Asp Lys Gln Pro Leu Thr
      1115                1120                1125

CAG TTT CAA TAC AGG CTG AAG GAA CTG GAC CAA ATC AAA TAT GTA CAC      1728
Gln Phe Gln Tyr Arg Leu Lys Glu Leu Asp Gln Ile Lys Tyr Val His
  1130                1135                1140

ATG TTT GGC CAT GTT GTG CAA ACC CAC CTG GAA CAC CAA GTG CCA GAT      1776
Met Phe Gly His Val Val Gln Thr His Leu Glu His Gln Val Pro Asp
1145                1150                1155                1160

ACT CCA GTT TTT TCT ACT GCG GGA GTT TCG AAA GTT TAC CCT CAG ATA      1824
Thr Pro Val Phe Ser Thr Ala Gly Val Ser Lys Val Tyr Pro Gln Ile
                1165                1170                1175

CTG TAG                                                             1830
Leu
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2253 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..2250

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
ATG AAG CGC GCA AGA CCG TCT GAA GAT ACC TTC AAC CCC GTG TAT CCA      48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
                615                 620                 625

TAT GAC ACG GAA ACC GGT CCT CCA ACT GTG CCT TTT CTT ACT CCT CCC      96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
            630                 635                 640

TTT GTA TCC CCC AAT GGG TTT CAA GAG AGT CCC CCT GGG GTA CTC TCT     144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
        645                 650                 655

TTG CGC CTA TCC GAA CCT CTA GTT ACC TCC AAT GGC ATG CTT GCG CTC     192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
    660                 665                 670

AAA ATG GGC AAC GGC CTC TCT CTG GAC GAG GCC GGC AAC CTT ACC TCC     240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
675                 680                 685                 690

CAA AAT GTA ACC ACT GTG AGC CCA CCT CTC AAA AAA ACC AAG TCA AAC     288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
                695                 700                 705

ATA AAC CTG GAA ATA TCT GCA CCC CTC ACA GTT ACC TCA GAA GCC CTA     336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
            710                 715                 720

ACT GTG GCT GCC GCC GCA CCT CTA ATG GTC GCG GGC AAC ACA CTC ACC     384
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
        725                 730                 735

ATG CAA TCA CAG GCC CCG CTA ACC GTG CAC GAC TCC AAA CTT AGC ATT     432
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
    740                 745                 750

GCC ACC CAA GGA CCC CTC ACA GTG TCA GAA GGA AAG CTA GCC CTG ACA     480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Thr
755                 760                 765                 770

AAA ACC TTA GTC TAT CCC ACC CTG TGG ACG GGG CCT GCT CCC GAG GCC     528
Lys Thr Leu Val Tyr Pro Thr Leu Trp Thr Gly Pro Ala Pro Glu Ala
                775                 780                 785

AAC GTC ACC TTC TCG GGG GAG AAT TCC CCA TCT GGC ATT CTC AGA CTG     576
Asn Val Thr Phe Ser Gly Glu Asn Ser Pro Ser Gly Ile Leu Arg Leu
            790                 795                 800

TGT CTC AGC AGA ACC GGG GGC ACG GTC ATT GGC ACC CTG TCT GTA CAA     624
Cys Leu Ser Arg Thr Gly Gly Thr Val Ile Gly Thr Leu Ser Val Gln
        805                 810                 815

GGT AGC CTC ACG AAC CCC AGT ACC GGT CAG ACC CTG GGC ATG AAC CTT     672
Gly Ser Leu Thr Asn Pro Ser Thr Gly Gln Thr Leu Gly Met Asn Leu
    820                 825                 830

TAC TTT GAC GCA GAC GGC AAT GTG CTG TCT GAG AGC AAC CTC GTC CGA     720
Tyr Phe Asp Ala Asp Gly Asn Val Leu Ser Glu Ser Asn Leu Val Arg
835                 840                 845                 850

GGG TCC TGG GGA ATG AAA GAC CAA GAT ACC CTG GTG ACT CCC ATT GCC     768
Gly Ser Trp Gly Met Lys Asp Gln Asp Thr Leu Val Thr Pro Ile Ala
                855                 860                 865
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AAT GGG CAG TAC CTG ATG CCC AAC CTC ACT GCA TAC CCT CGC CTC ATA      816
Asn Gly Gln Tyr Leu Met Pro Asn Leu Thr Ala Tyr Pro Arg Leu Ile
            870                 875                 880

CAG ACC CTA ACT TCC AGC TAC ATT TAC ACA CAA GCG CAC CTT GAC CAC      864
Gln Thr Leu Thr Ser Ser Tyr Ile Tyr Thr Gln Ala His Leu Asp His
        885                 890                 895

AAT AAC AGT GTG GTG GAC ATC AAG ATA GGG CTC AAC ACA GAC CTG AGG      912
Asn Asn Ser Val Val Asp Ile Lys Ile Gly Leu Asn Thr Asp Leu Arg
    900                 905                 910

CCC ACT GCG GCC TAC GGC CTA AGC TTT ACC ATG ACC TTC ACT AAC TCT      960
Pro Thr Ala Ala Tyr Gly Leu Ser Phe Thr Met Thr Phe Thr Asn Ser
915                 920                 925                 930

CCC CCC ACC TCA TTT GGT ACC GAC CTG GTG CAA TTT GGC TAC CTG GGT     1008
Pro Pro Thr Ser Phe Gly Thr Asp Leu Val Gln Phe Gly Tyr Leu Gly
                935                 940                 945

CAG GAT AGC TCC CCC TCC TTC CTG AGA GAA CTT CCC CTT GCA TCC GAG     1056
Gln Asp Ser Ser Pro Ser Phe Leu Arg Glu Leu Pro Leu Ala Ser Glu
            950                 955                 960

GCG GGC TAC TTT GGC AAA CTG GCA GCT GCC TCT GAG GAA ATG CCA GCC     1104
Ala Gly Tyr Phe Gly Lys Leu Ala Ala Ala Ser Glu Glu Met Pro Ala
        965                 970                 975

CCT CCT GAG GCC CAG ACG CAG GAC CAA GCA GCT GAG GAG CCC CCG GCT     1152
Pro Pro Glu Ala Gln Thr Gln Asp Gln Ala Ala Glu Glu Pro Pro Ala
    980                 985                 990

CCT GCT GAG GCT GAG GCC CCC GCT CCT GCT GAG GCT GAG GCT GAG GCT     1200
Pro Ala Glu Ala Glu Ala Pro Ala Pro Ala Glu Ala Glu Ala Glu Ala
995                 1000                1005                1010

GAA CCG CCC CGA AAA CCC CCT AGG GGT GAC CTG GCC GCC CTA TAC AAT     1248
Glu Pro Pro Arg Lys Pro Pro Arg Gly Asp Leu Ala Ala Leu Tyr Asn
                1015                1020                1025

AGG GTC CAC AGC GAC ACC CGC GCA GAG GAC ACA CCA ACC AGC CCC GAG     1296
Arg Val His Ser Asp Thr Arg Ala Glu Asp Thr Pro Thr Ser Pro Glu
            1030                1035                1040

TTG GTC ACA ACC TTG CCA GAC CCC TTT GTC CTC CCC CTA CCC GAC GGA     1344
Leu Val Thr Thr Leu Pro Asp Pro Phe Val Leu Pro Leu Pro Asp Gly
        1045                1050                1055

GTC CCA ACC GGT GCG AGC ATT GTG TTG GAA GGT ACC CTC ACA CCC TCC     1392
Val Pro Thr Gly Ala Ser Ile Val Leu Glu Gly Thr Leu Thr Pro Ser
    1060                1065                1070

GCT GTG TTT TTT ACC CTG GAT CTG GTG ACC GGG CCC GCC AGT CTG GCG     1440
Ala Val Phe Phe Thr Leu Asp Leu Val Thr Gly Pro Ala Ser Leu Ala
1075                1080                1085                1090

CTG CAC TTT AAC GTG CGC CTC CCA CTG GAA GGC GAA AAG CAC ATT GTG     1488
Leu His Phe Asn Val Arg Leu Pro Leu Glu Gly Glu Lys His Ile Val
                1095                1100                1105

TGC AAC TCC AGA GAG GGT AGC AGC AAC TGG GGC GAA GAA GTA AGA CCG     1536
Cys Asn Ser Arg Glu Gly Ser Ser Asn Trp Gly Glu Glu Val Arg Pro
            1110                1115                1120

CAG GAG TTC CCC TTT GAA AGG GAA AAG CCA TTC GTC CTG GTC ATT GTC     1584
Gln Glu Phe Pro Phe Glu Arg Glu Lys Pro Phe Val Leu Val Ile Val
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        1125                1130                1135

ATC CAA AGT GAC ACA TAC CAG ATC ACT GTG AAC GGG AAG CCT CTG GTG      1632
Ile Gln Ser Asp Thr Tyr Gln Ile Thr Val Asn Gly Lys Pro Leu Val
    1140                1145                1150

GAT TTT CCA CAG AGA CTA CAG GGC ATT ACC CGT GCC TCC CTA TCC GGA      1680
Asp Phe Pro Gln Arg Leu Gln Gly Ile Thr Arg Ala Ser Leu Ser Gly
1155                1160                1165                1170

GAC CTT GTG TTT ACC CGG TTG ACA ATG TAC CCA CCC GGA GAC CCC CGT      1728
Asp Leu Val Phe Thr Arg Leu Thr Met Tyr Pro Pro Gly Asp Pro Arg
                1175                1180                1185

CCC ACA ACC TTG TTA CCA CCC CCC GCA GCT CCC CTG GAC GTA ATC CCA      1776
Pro Thr Thr Leu Leu Pro Pro Pro Ala Ala Pro Leu Asp Val Ile Pro
            1190                1195                1200

GAT GCC TAT GTG CTC AAT CTG CCC ACC GGA CTG ACG CCT AGA ACA CTC      1824
Asp Ala Tyr Val Leu Asn Leu Pro Thr Gly Leu Thr Pro Arg Thr Leu
        1205                1210                1215

CTC ACC GTC ACG GGA ACC CCC ACG CCC CTC GCC GAA TTT TTT ATT GTG      1872
Leu Thr Val Thr Gly Thr Pro Thr Pro Leu Ala Glu Phe Phe Ile Val
    1220                1225                1230

AAT CTG GTC TAC GAT TTA CAC TAT GAT TCC AAA AAT GTG GCC CTC CAC      1920
Asn Leu Val Tyr Asp Leu His Tyr Asp Ser Lys Asn Val Ala Leu His
1235                1240                1245                1250

TTT AAT GTC GGC TTC ACC TCT GAC AGC AAA GGC CAC ATC GCC TGC AAT      1968
Phe Asn Val Gly Phe Thr Ser Asp Ser Lys Gly His Ile Ala Cys Asn
                1255                1260                1265

GCC AGA ATG AAT GGC ACA TGG GGA AGT GAA ATC ACA GTG TCT GAT TTC      2016
Ala Arg Met Asn Gly Thr Trp Gly Ser Glu Ile Thr Val Ser Asp Phe
            1270                1275                1280

CCC TTT CAA AGG GGA AAA CCC TTC ACT CTG CAG ATT CTC ACC AGA GAG      2064
Pro Phe Gln Arg Gly Lys Pro Phe Thr Leu Gln Ile Leu Thr Arg Glu
        1285                1290                1295

GCA GAC TTC CAA GTC CTC GTA GAT AAA CAA CCT TTA ACC CAG TTT CAA      2112
Ala Asp Phe Gln Val Leu Val Asp Lys Gln Pro Leu Thr Gln Phe Gln
    1300                1305                1310

TAC AGG CTG AAG GAA CTG GAC CAA ATC AAA TAT GTA CAC ATG TTT GGC      2160
Tyr Arg Leu Lys Glu Leu Asp Gln Ile Lys Tyr Val His Met Phe Gly
1315                1320                1325                1330

CAT GTT GTG CAA ACC CAC CTG GAA CAC CAA GTG CCA GAT ACT CCA GTT      2208
His Val Val Gln Thr His Leu Glu His Gln Val Pro Asp Thr Pro Val
                1335                1340                1345

TTT TCT ACT GCG GGA GTT TCG AAA GTT TAC CCT CAG ATA CTG TAG          2253
Phe Ser Thr Ala Gly Val Ser Lys Val Tyr Pro Gln Ile Leu
            1350                1355                1360
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 795 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..792

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
ATG GCG CTC CTG CTG TGC TTC GTG CTC CTG TGC GGA GTA GTG GAT TTC        48
Met Ala Leu Leu Leu Cys Phe Val Leu Leu Cys Gly Val Val Asp Phe
          755                 760                 765

GCC AGA AGT TTG AGT ATC ACT ACT CCT GAA GAG ATG ATT GAA AAA GCC        96
Ala Arg Ser Leu Ser Ile Thr Thr Pro Glu Glu Met Ile Glu Lys Ala
      770                 775                 780

AAA GGG GAA ACT GCC TAT CTG CCG TGC AAA TTT ACG CTT AGT CCC GAA       144
Lys Gly Glu Thr Ala Tyr Leu Pro Cys Lys Phe Thr Leu Ser Pro Glu
  785                 790                 795

GAC CAG GGA CCG CTG GAC ATC GAG TGG CTG ATA TCA CCA GCT GAT AAT       192
Asp Gln Gly Pro Leu Asp Ile Glu Trp Leu Ile Ser Pro Ala Asp Asn
800                 805                 810                 815

CAG AAG GTG GAT CAA GTG ATT ATT TTA TAT TCT GGA GAC AAA ATT TAT       240
Gln Lys Val Asp Gln Val Ile Ile Leu Tyr Ser Gly Asp Lys Ile Tyr
                820                 825                 830

GAT GAC TAC TAT CCA GAT CTG AAA GGC CGA GTA CAT TTT ACG AGT AAT       288
Asp Asp Tyr Tyr Pro Asp Leu Lys Gly Arg Val His Phe Thr Ser Asn
          835                 840                 845

GAT CTC AAA TCT GGT GAT GCA TCA ATA AAT GTA ACG AAT TTA CAA CTG       336
Asp Leu Lys Ser Gly Asp Ala Ser Ile Asn Val Thr Asn Leu Gln Leu
      850                 855                 860

TCA GAT ATT GGC ACA TAT CAG TGC AAA GTG AAA AAA GCT CCT GGT GTT       384
Ser Asp Ile Gly Thr Tyr Gln Cys Lys Val Lys Lys Ala Pro Gly Val
  865                 870                 875

GCA AAT AAG AAG ATT CAT CTG GTA GTT CTT GTT AAG CCT TCA GGT GCG       432
Ala Asn Lys Lys Ile His Leu Val Val Leu Val Lys Pro Ser Gly Ala
880                 885                 890                 895

AGA TGT TAC GTT GAT GGA TCT GAA GAA ATT GGA AGT GAC TTT AAG ATA       480
Arg Cys Tyr Val Asp Gly Ser Glu Glu Ile Gly Ser Asp Phe Lys Ile
                900                 905                 910

AAA TGT GAA CCA AAA GAA GGT TCA CTT CCA TTA CAG TAT GAG TGG CAA       528
Lys Cys Glu Pro Lys Glu Gly Ser Leu Pro Leu Gln Tyr Glu Trp Gln
          915                 920                 925

AAA TTG TCT GAC TCA CAG AAA ATG CCC ACT TCA TGG TTA GCA GAA ATG       576
Lys Leu Ser Asp Ser Gln Lys Met Pro Thr Ser Trp Leu Ala Glu Met
      930                 935                 940

ACT TCA TCT GTT ATA TCT GTA AAA AAT GCC TCT TCT GAG TAC TCT GGG       624
Thr Ser Ser Val Ile Ser Val Lys Asn Ala Ser Ser Glu Tyr Ser Gly
  945                 950                 955

ACA TAC AGC TGT ACA GTC AGA AAC AGA GTG GGC TCT GAT CAG TGC CTG       672
Thr Tyr Ser Cys Thr Val Arg Asn Arg Val Gly Ser Asp Gln Cys Leu
960                 965                 970                 975

TTG CGT CTA AAC GTT GTC CCT CCT TCA AAT AAA GCT GGA TCT GGA TCC       720
Leu Arg Leu Asn Val Val Pro Pro Ser Asn Lys Ala Gly Ser Gly Ser
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                980                 985                 990

GGC TCA GGG TCT ACT AGT GGG GCC CAG CCG GCC CTG CAG GCG GCC GCA      768
Gly Ser Gly Ser Thr Ser Gly Ala Gln Pro Ala Leu Gln Ala Ala Ala
           995                1000                1005

GAC TAT AAA GAT GAC GAC GAT AAG TGA                                  795
Asp Tyr Lys Asp Asp Asp Asp Lys
       1010                1015
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 834 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..831

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
ATG GCG CTC CTG CTG TGC TTC GTG CTC CTG TGC GGA GTA GTG GAT TTC       48
Met Ala Leu Leu Leu Cys Phe Val Leu Leu Cys Gly Val Val Asp Phe
                270                 275                 280

GCC AGA AGT TTG AGT ATC ACT ACT CCT GAA GAG ATG ATT GAA AAA GCC       96
Ala Arg Ser Leu Ser Ile Thr Thr Pro Glu Glu Met Ile Glu Lys Ala
           285                 290                 295

AAA GGG GAA ACT GCC TAT CTG CCG TGC AAA TTT ACG CTT AGT CCC GAA      144
Lys Gly Glu Thr Ala Tyr Leu Pro Cys Lys Phe Thr Leu Ser Pro Glu
       300                 305                 310

GAC CAG GGA CCG CTG GAC ATC GAG TGG CTG ATA TCA CCA GCT GAT AAT      192
Asp Gln Gly Pro Leu Asp Ile Glu Trp Leu Ile Ser Pro Ala Asp Asn
   315                 320                 325

CAG AAG GTG GAT CAA GTG ATT ATT TTA TAT TCT GGA GAC AAA ATT TAT      240
Gln Lys Val Asp Gln Val Ile Ile Leu Tyr Ser Gly Asp Lys Ile Tyr
330                 335                 340                 345

GAT GAC TAC TAT CCA GAT CTG AAA GGC CGA GTA CAT TTT ACG AGT AAT      288
Asp Asp Tyr Tyr Pro Asp Leu Lys Gly Arg Val His Phe Thr Ser Asn
                350                 355                 360

GAT CTC AAA TCT GGT GAT GCA TCA ATA AAT GTA ACG AAT TTA CAA CTG      336
Asp Leu Lys Ser Gly Asp Ala Ser Ile Asn Val Thr Asn Leu Gln Leu
           365                 370                 375

TCA GAT ATT GGC ACA TAT CAG TGC AAA GTG AAA AAA GCT CCT GGT GTT      384
Ser Asp Ile Gly Thr Tyr Gln Cys Lys Val Lys Lys Ala Pro Gly Val
       380                 385                 390

GCA AAT AAG AAG ATT CAT CTG GTA GTT CTT GTT AAG CCT TCA GGT GCG      432
Ala Asn Lys Lys Ile His Leu Val Val Leu Val Lys Pro Ser Gly Ala
   395                 400                 405

AGA TGT TAC GTT GAT GGA TCT GAA GAA ATT GGA AGT GAC TTT AAG ATA      480
Arg Cys Tyr Val Asp Gly Ser Glu Glu Ile Gly Ser Asp Phe Lys Ile
410                 415                 420                 425
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AAA TGT GAA CCA AAA GAA GGT TCA CTT CCA TTA CAG TAT GAG TGG CAA      528
Lys Cys Glu Pro Lys Glu Gly Ser Leu Pro Leu Gln Tyr Glu Trp Gln
                430                 435                 440

AAA TTG TCT GAC TCA CAG AAA ATG CCC ACT TCA TGG TTA GCA GAA ATG      576
Lys Leu Ser Asp Ser Gln Lys Met Pro Thr Ser Trp Leu Ala Glu Met
            445                 450                 455

ACT TCA TCT GTT ATA TCT GTA AAA AAT GCC TCT TCT GAG TAC TCT GGG      624
Thr Ser Ser Val Ile Ser Val Lys Asn Ala Ser Ser Glu Tyr Ser Gly
        460                 465                 470

ACA TAC AGC TGT ACA GTC AGA AAC AGA GTG GGC TCT GAT CAG TGC CTG      672
Thr Tyr Ser Cys Thr Val Arg Asn Arg Val Gly Ser Asp Gln Cys Leu
    475                 480                 485

TTG CGT CTA AAC GTT GTC CCT CCT TCA AAT AAA GCT GGA TCT GGA TCC      720
Leu Arg Leu Asn Val Val Pro Pro Ser Asn Lys Ala Gly Ser Gly Ser
490                 495                 500                 505

GGC TCA GGG TCT ACT AGA GCC TGC GAC TGT CGC GGC GAT TGT TTT TGC      768
Gly Ser Gly Ser Thr Arg Ala Cys Asp Cys Arg Gly Asp Cys Phe Cys
                510                 515                 520

GGT ACT AGT GGG GCC CAG CCG GCC CTG CAG GCG GCC GCA GAC TAT AAA      816
Gly Thr Ser Gly Ala Gln Pro Ala Leu Gln Ala Ala Ala Asp Tyr Lys
            525                 530                 535

GAT GAC GAC GAT AAG TGA                                             834
Asp Asp Asp Asp Lys
        540
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1194 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..1191

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
ATG GCG CTC CTG CTG TGC TTC GTG CTC CTG TGC GGA GTA GTG GAT TTC       48
Met Ala Leu Leu Leu Cys Phe Val Leu Leu Cys Gly Val Val Asp Phe
    280                 285                 290

GCC AGA AGT TTG AGT ATC ACT ACT CCT GAA GAG ATG ATT GAA AAA GCC       96
Ala Arg Ser Leu Ser Ile Thr Thr Pro Glu Glu Met Ile Glu Lys Ala
295                 300                 305                 310

AAA GGG GAA ACT GCC TAT CTG CCG TGC AAA TTT ACG CTT AGT CCC GAA      144
Lys Gly Glu Thr Ala Tyr Leu Pro Cys Lys Phe Thr Leu Ser Pro Glu
                315                 320                 325

GAC CAG GGA CCG CTG GAC ATC GAG TGG CTG ATA TCA CCA GCT GAT AAT      192
Asp Gln Gly Pro Leu Asp Ile Glu Trp Leu Ile Ser Pro Ala Asp Asn
            330                 335                 340

CAG AAG GTG GAT CAA GTG ATT ATT TTA TAT TCT GGA GAC AAA ATT TAT      240
Gln Lys Val Asp Gln Val Ile Ile Leu Tyr Ser Gly Asp Lys Ile Tyr
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        345                 350                 355

GAT GAC TAC TAT CCA GAT CTG AAA GGC CGA GTA CAT TTT ACG AGT AAT      288
Asp Asp Tyr Tyr Pro Asp Leu Lys Gly Arg Val His Phe Thr Ser Asn
    360                 365                 370

GAT CTC AAA TCT GGT GAT GCA TCA ATA AAT GTA ACG AAT TTA CAA CTG      336
Asp Leu Lys Ser Gly Asp Ala Ser Ile Asn Val Thr Asn Leu Gln Leu
375                 380                 385                 390

TCA GAT ATT GGC ACA TAT CAG TGC AAA GTG AAA AAA GCT CCT GGT GTT      384
Ser Asp Ile Gly Thr Tyr Gln Cys Lys Val Lys Lys Ala Pro Gly Val
                395                 400                 405

GCA AAT AAG AAG ATT CAT CTG GTA GTT CTT GTT AAG CCT TCA GGT GCG      432
Ala Asn Lys Lys Ile His Leu Val Val Leu Val Lys Pro Ser Gly Ala
            410                 415                 420

AGA TGT TAC GTT GAT GGA TCT GAA GAA ATT GGA AGT GAC TTT AAG ATA      480
Arg Cys Tyr Val Asp Gly Ser Glu Glu Ile Gly Ser Asp Phe Lys Ile
        425                 430                 435

AAA TGT GAA CCA AAA GAA GGT TCA CTT CCA TTA CAG TAT GAG TGG CAA      528
Lys Cys Glu Pro Lys Glu Gly Ser Leu Pro Leu Gln Tyr Glu Trp Gln
    440                 445                 450

AAA TTG TCT GAC TCA CAG AAA ATG CCC ACT TCA TGG TTA GCA GAA ATG      576
Lys Leu Ser Asp Ser Gln Lys Met Pro Thr Ser Trp Leu Ala Glu Met
455                 460                 465                 470

ACT TCA TCT GTT ATA TCT GTA AAA AAT GCC TCT TCT GAG TAC TCT GGG      624
Thr Ser Ser Val Ile Ser Val Lys Asn Ala Ser Ser Glu Tyr Ser Gly
                475                 480                 485

ACA TAC AGC TGT ACA GTC AGA AAC AGA GTG GGC TCT GAT CAG TGC CTG      672
Thr Tyr Ser Cys Thr Val Arg Asn Arg Val Gly Ser Asp Gln Cys Leu
            490                 495                 500

TTG CGT CTA AAC GTT GTC CCT CCT TCA AAT AAA GCT GGA TCT GGA TCC      720
Leu Arg Leu Asn Val Val Pro Pro Ser Asn Lys Ala Gly Ser Gly Ser
        505                 510                 515

GGC TCA GGG TCT ACT AGA GGA GGT GGT GCA TCA AGG GTC TCG GCG CTC      768
Gly Ser Gly Ser Thr Arg Gly Gly Gly Ala Ser Arg Val Ser Ala Leu
    520                 525                 530

GAG AAG ACG TCT CAA ATA CAC TCT GAT ACT ATC CTC CGG ATC ACC CAG      816
Glu Lys Thr Ser Gln Ile His Ser Asp Thr Ile Leu Arg Ile Thr Gln
535                 540                 545                 550

GGA CTC GAT GAT GCA AAC AAA CGA ATC ATC GCT CTT GAG CAA AGT CGG      864
Gly Leu Asp Asp Ala Asn Lys Arg Ile Ile Ala Leu Glu Gln Ser Arg
                555                 560                 565

GAT GAC TTG GTT GCA TCA GTC AGT GAT GCT CAA CTT GCA ATC TCC AGA      912
Asp Asp Leu Val Ala Ser Val Ser Asp Ala Gln Leu Ala Ile Ser Arg
            570                 575                 580

TTG GAA AGC TCT ATC GGA GCC CTC CAA ACA GTT GTC AAT GGA CTT GAT      960
Leu Glu Ser Ser Ile Gly Ala Leu Gln Thr Val Val Asn Gly Leu Asp
        585                 590                 595

TCG AGT GTT ACC CAG TTG GGT GCT CGA GTG GGA CAA CTT GAG ACA GGA     1008
Ser Ser Val Thr Gln Leu Gly Ala Arg Val Gly Gln Leu Glu Thr Gly
    600                 605                 610
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CTT GCA GAC GTA CGC GTT GAT CAC GAC AAT CTC GTT GCG AGA GTG GAT      1056
Leu Ala Asp Val Arg Val Asp His Asp Asn Leu Val Ala Arg Val Asp
615                 620                 625                 630

ACT GCA GAA CGT AAC ATT GGA TCA TTG ACC ACT GAG CTA TCA ACT CTG      1104
Thr Ala Glu Arg Asn Ile Gly Ser Leu Thr Thr Glu Leu Ser Thr Leu
                635                 640                 645

ACG TTA CGA GTA ACA TCC ATA CAA GCG GAT TTC GAA TCT AGG ACT AGT      1152
Thr Leu Arg Val Thr Ser Ile Gln Ala Asp Phe Glu Ser Arg Thr Ser
            650                 655                 660

ATG CAG GCG GCC GCA GAC TAT AAA GAT GAC GAC GAT AAG TGA              1194
Met Gln Ala Ala Ala Asp Tyr Lys Asp Asp Asp Asp Lys
        665                 670                 675
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1743 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..1743

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
ATG AAG CGC GCA AGA CCG TCT GAA GAT ACC TTC AAC CCC GTG TAT CCA       48
Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
  1               5                  10                  15

TAT GAC ACG GAA ACC GGT CCT CCA ACT GTG CCT TTT CTT ACT CCT CCC       96
Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
             20                  25                  30

TTT GTA TCC CCC AAT GGG TTT CAA GAG AGT CCC CCT GGG GTA CTC TCT      144
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
         35                  40                  45

TTG CGC CTA TCC GAA CCT CTA GTT ACC TCC AAT GGC ATG CTT GCG CTC      192
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
     50                  55                  60

AAA ATG GGC AAC GGC CTC TCT CTG GAC GAG GCC GGC AAC CTT ACC TCC      240
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
 65                  70                  75                  80

CAA AAT GTA ACC ACT GTG AGC CCA CCT CTC AAA AAA ACC AAG TCA AAC      288
Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
                85                  90                  95

ATA AAC CTG GAA ATA TCT GCA CCC CTC ACA GTT ACC TCA GAA GCC CTA      336
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu
            100                 105                 110

ACT GTG GCT GCC GCC GCA CCT CTA ATG GTC GCG GGC AAC ACA CTC ACC      384
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
        115                 120                 125

ATG CAA TCA CAG GCC CCG CTA ACC GTG CAC GAC TCC AAA CTT AGC ATT      432
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile
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    130                 135                 140

GCC ACC CAA GGA CCC CTC ACA GTG TCA GAA GGA AAG CTA GCC CTG CAA      480
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
145                 150                 155                 160

ACA TCA GGC CCC CTC ACC ACC ACC GAT AGC AGT ACC CTT ACT ATC ACT      528
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr
                165                 170                 175

GCC TCA CCC CCT CTA ACT ACT GCC ACT GGT AGC TTG GGC ATT GAC TTG      576
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
            180                 185                 190

AAA GAG CCC ATT TAT ACA CAA AAT GGA AAA CTA GGA CTA AAG TAC GGG      624
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
        195                 200                 205

GCT CCT TTG CAT GTA ACA GAC GAC CTA AAC ACT TTG ACC GTA GCA ACT      672
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
    210                 215                 220

GGT CCA GGT GTG ACT ATT AAT AAT ACT TCC TTG CAA ACT AAA GTT ACT      720
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr
225                 230                 235                 240

GGA GCC TTG GGT TTT GAT TCA CAA GGC AAT ATG CAA CTT AAT GTA GCA      768
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
                245                 250                 255

GGA GGA CTA AGG ATT GAT TCT CAA AAC AGA CGC CTT ATA CTT GAT GTT      816
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
            260                 265                 270

AGT TAT CCG TTT GAT GCT CAA AAC CAA CTA AAT CTA AGA CTA GGA CAG      864
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
        275                 280                 285

GGC CCT CTT TTT ATA AAC TCA GCC CAC AAC TTG GAT ATT AAC TAC AAC      912
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
    290                 295                 300

AAA GGC CTT TAC TTG TTT ACA GCT TCA AAC AAT TCC AAA AAG CTT GAG      960
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305                 310                 315                 320

GTT AAC CTA AGC ACT GCC AAG GGG TTG ATG TTT GAC GCT ACA GCC ATA     1008
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
                325                 330                 335

GCC ATT AAT GCA GGA GAT GGG CTT GAA TTT GGT TCA CCT AAT GCA CCA     1056
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
            340                 345                 350

AAC ACA AAT CCC CTC AAA ACA AAA ATT GGC CAT GGC CTA GAA TTT GAT     1104
Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
        355                 360                 365

TCA AAC AAG GCT ATG GTT CCT AAA CTA GGA ACT GGC CTT AGT TTT GAC     1152
Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
    370                 375                 380

AGC ACA GGT GCC ATT ACA GTA GGA AAC AAA AAT AAT GAT AAG CTA ACT     1200
Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385                 390                 395                 400
```

```
TTG TGG ACC ACA CCA GCT CCA TCT CCT AAC TGT AGA CTA AAT GCA GAG      1248
Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
                405                 410                 415

AAA GAT GCT AAA CTC ACT TTG GTC TTA ACA AAA TGT GGC AGT CAA ATA      1296
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
            420                 425                 430

CTT GCT ACA GTT TCA GTT TTG GCT GTT AAA GGC AGT TTG GCT CCA ATA      1344
Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
        435                 440                 445

TCT GGA ACA GTT CAA AGT GCT CAT CTT ATT ATA AGA TTT GAC GAA AAT      1392
Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
    450                 455                 460

GGA GTG CTA CTA AAC AAT TCC TTC CTG GAC CCA GAA TAT TGG AAC TTT      1440
Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
465                 470                 475                 480

AGA AAT GGA GAT CTT ACT GAA GGC ACA GCC TAT ACA AAC GCT GTT GGA      1488
Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
                485                 490                 495

TTT ATG CCT AAC CTA TCA GCT TAT CCA AAA TCT CAC GGT AAA ACT GCC      1536
Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
            500                 505                 510

AAA AGT AAC ATT GTC AGT CAA GTT TAC TTA AAC GGA GAC AAA ACT AAA      1584
Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
        515                 520                 525

CCT GTA ACA CTA ACC ATT ACA CTA AAC GGT ACA CAG GAA ACA GGA GAC      1632
Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp
    530                 535                 540

ACA ACT CCA AGT GCA TAC TCT ATG TCA TTT TCA TGG GAC TGG TCT GGC      1680
Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly
545                 550                 555                 560

CAC AAC TAC ATT AAT GAA ATA TTT GCC ACA TCC TCT TAC ACT TTT TCA      1728
His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser
                565                 570                 575

TAC ATT GCC CAA GAA                                                 1743
Tyr Ile Ala Gln Glu
            580
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGCATGCATA CTAGTCCTAG ATTCGAAATC CGCTTG 36

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCTCTAGAGG AGGTGGTGCA TCAAGGGTCT CGGCGCTC 38

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCGGATCCCT ACAGTATCTG AGGGTAAAC 29

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGGCACCATG GCGAAGATGG AGCTTTGTCC C 31

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Thr Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Arg Lys Lys Lys Arg Lys Lys Lys

1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Pro Lys Ala Arg Arg Pro Ala Gly Arg Thr Trp Ala Gln Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Arg Pro Ile Asp Asp Phe Asp Gln Gly Trp Gly Pro Ile Thr Tyr
1 5 10 15

WHAT IS CLAIMED IS:

1. A trimer comprising three monomers, each of said monomers having an amino terminus of an adenoviral fiber protein and each of said monomers having a trimerization domain, wherein said trimer exhibits reduced affinity for a native substrate as compared to a native adenoviral fiber trimer.

2. The trimer of claim 1, which is not a ligand for a native mammalian cell-surface binding site.

3. The trimer of claim 1 or 2, wherein said trimerization domain of at least one of said monomers is an adenoviral fiber knob domain lacking a native substrate-binding amino acid.

4. The trimer of claim 3, wherein said native substrate-binding amino acid is within a β-sheet.

5. The trimer of claim 3, wherein said native substrate-binding amino acid is within a loop connecting two β-sheets.

6. The trimer of any of claims 3-5, wherein said native substrate-binding amino acid is substituted with a non-native residue.

7. The trimer of claim 6, wherein said non-native residue has a charge different from said native substrate-binding amino acid.

8. The trimer of claim 6 or 7, wherein said non-native residue is has a greater molecular weight than said native substrate-binding amino acid.

9. The trimer of any of claims 1-8, which comprises chimeric adenoviral fiber polypeptides of said three monomers.

10. The trimer of any of claims 1-9, wherein at least one of said trimerization domains is not a mammalian adenoviral trimerization domain.

11. The trimer of any of claims 1-10, wherein each of said trimerization domains is derived from the sigma-1 protein of reovirus.

12. The trimer of any of claims 1-10, wherein each of said trimerization domains comprises a modified leucine-zipper motif.

13. The trimer of any of claims 1-12, wherein at least one of said three monomers comprises a non-native polypeptide interfering with the binding of said trimer to its native cell-surface binding site.

14. A composition of matter comprising a trimer of any of claims 1-13 and an adenoviral penton base.

15. The composition of claim 14, wherein said penton base comprises a non-native ligand.

16. An adenovirus comprising the trimer of any of claims 1-13.

17. The adenovirus of claim 16, which does not productively infect 293 cells.

18. The adenovirus of claim 16 or 17, comprising a non-adenoviral ligand.

19. The adenovirus of claim 18, wherein said ligand binds a substrate other than a native mammalian adenoviral receptor.

20. The adenovirus of claim 18 or 19, wherein said ligand binds a substrate other than a native cell-surface protein.

21. The adenovirus of claim 19 or 20, wherein said substrate is present on the surface of a cell.

22. The adenovirus of claim 19 or 20, wherein said substrate is present within an affinity column.

23. The adenovirus of claim 19 or 20, wherein said substrate is present on a blood-borne molecule.

24. A cell line expressing a non-natural cell-surface receptor to which an adenovirus having a ligand for said receptor binds.

25. A method of propagating an adenovirus comprising infecting a cell line of claim 24 with an adenovirus, maintaining said cell line, and recovering the adenoviruses produced within said cell line.

26. A method of purifying an adenovirus having a ligand for a substrate from a composition comprising said adenovirus, wherein said method comprises exposing said composition to said substrate such that said adenovirus selectively binds to said substrate, separating said substrate from said composition without removing said adenovirus from said substrate, and eluting said adenovirus from said substrate.

27. A method of inactivating in a fluid an adenovirus having a ligand recognizing a fluid-borne substrate by exposing said virus to said substrate such that said ligand binds said substrate, thereby adsorbing said virus from said fluid.

28. The method of claim 27, wherein said fluid is blood or lymph

29. A chimeric blocking protein comprising a substrate for an adenovirus fiber.

30. The chimeric blocking protein of claim 29, wherein said substrate is the extracellular domain of the CAR cell-surface protein.

31. The chimeric blocking protein of claim 29 or 30, further comprising a ligand.

32. The chimeric blocking protein of claim 31, wherein said ligand recognizes a substrate present on a cell surface binding site.

33. A method of interfering with adenoviral targeting comprising incubating an adenovirus with the chimeric blocking protein of any of claims 29-32 in a solution such that said chimeric blocking protein binds the fiber of the adenovirus.

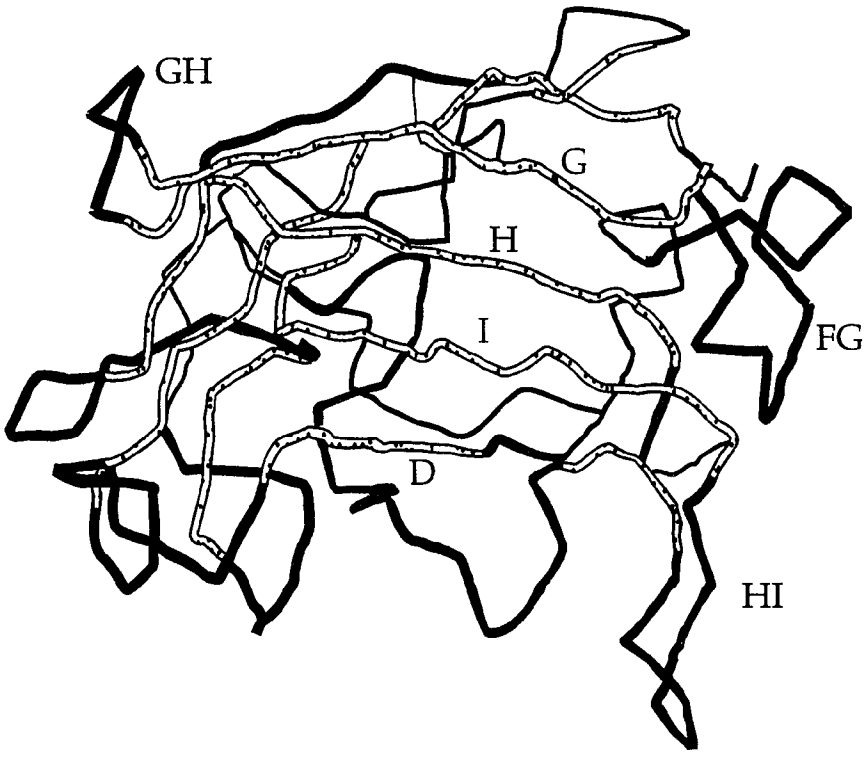


FIG. 1A

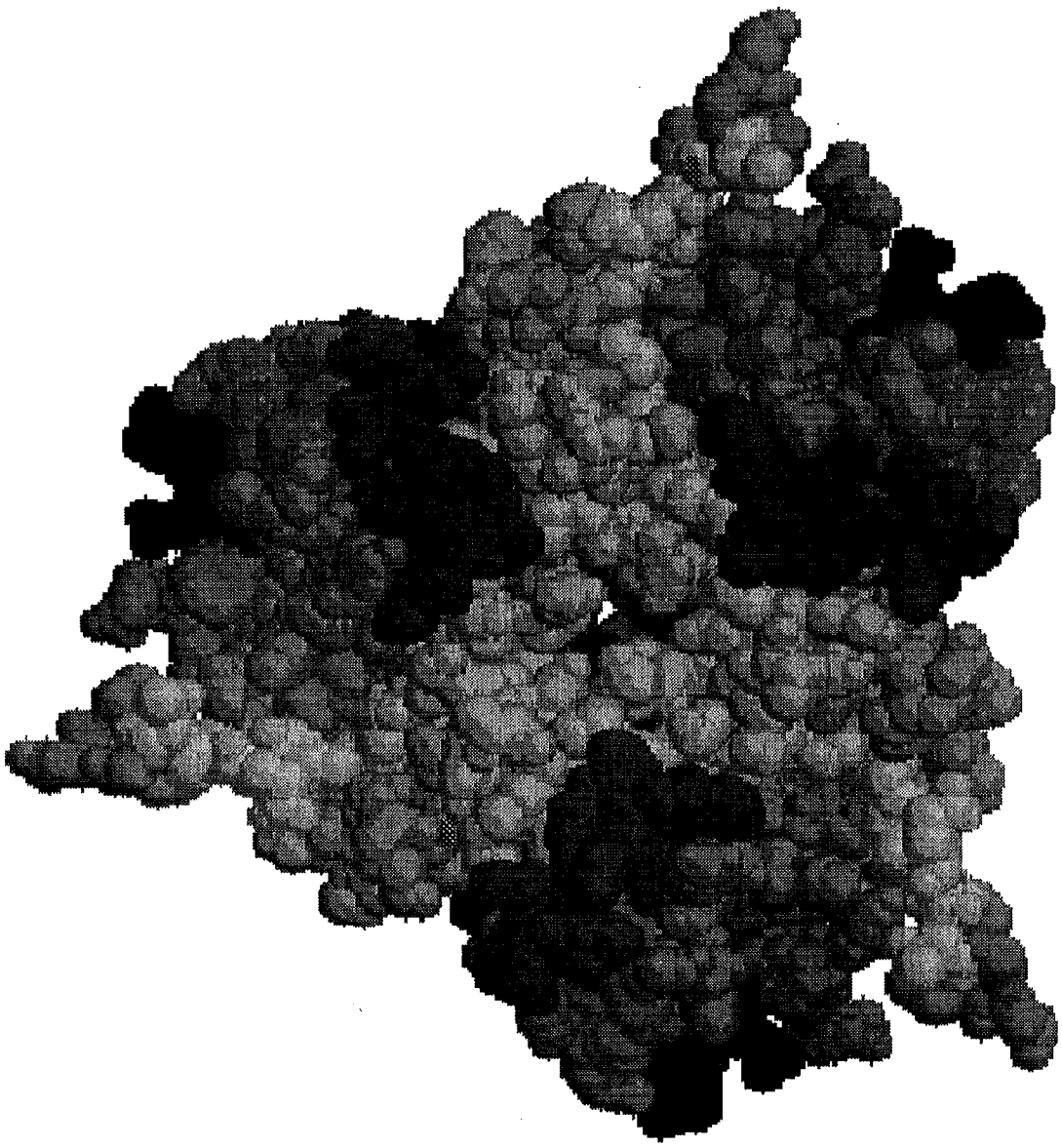


FIG. 1B

```
     NKNE---DKL--TLWTTPDPSPNCRID---QDKDAKLTLVLTKCGSQVLA  Majority
                460       470       480       490       500
393  NKND---DKL--TLWTTPDPSPNCRIH---SDHDCKFTLVLTKCGSQVLA  Fiber2
393  NKNN---DKL--TLWTTPAPSPNCRLN---AEKDAKLTLVLTKCGSQILA  Fiber5
241  NKDY---DKL--TLWTTPDPSPNCQIL---AENDAKLTLCLTKCDSQILA  Fiber4
174  NKKE---DKR--TLWTTPDTSPNCRID---QDKDSKLSLVLTKCGSQILA  Fiber8
174  NKKE---DKR--TLWTTPDTSPNCKID---QDKDSKLTLVLTKCGSQILA  Fiber9
365  PTTTTP---T--TLWTTADPSPNATFY---ESLDAKVWLVLVKCNGMVNG  Fiber40(long)
220  NRIQTRSVTSLTTIWSIS-PTPNCSIY---ETQDANLFLCLTKNGAHVLG  Fiber40(short)
399  SSSHTPYDPL--TLWTTPDPPPNCSLI---QELDAKLTLCLTKNGSIVNG  Fiber12
130  NN----------TLWTGPKPEANCIIEYGKQNPDSKLTLILVKNGGIVNG  Fiber3
```

FIG. 2

```
                                                                  Majority
TVSVLGVK-GLLKIINGTVDSA----------TVKLRFDANGVLLESS
                  510       520       530       540       550
435 TVAALAVS-GDLSSMTGTVASV----------SIFLRFDQNGVLMENS  Fiber2
435 TVSVLAVK-GSLAPISGTVQSA----------HLIIRFDENGVLLNNS  Fiber5
283 TVSVLVVRSGNLNPITGTVSSA----------QVFLRFDANGVLLTEH  Fiber4
216 NVS-LIVVAGRYKIINNNTNPALKGF----------TIKLLFDENGVLMESS  Fiber8
216 NVS-LIVVDGKYKIINNNTQPALKGF----------TIKLLFDENGVLMESS  Fiber9
407 TISIKAQKGTLLK--PTASFISFVMYFYSDGTWRKNYPVFDNEGILANSA  Fiber40(long)
266 TITIKGLK-GALREMNDNA----------LSVKLPFDNQGNLL-NC  Fiber40(short)
444 IVSLVGVKGNLLNIQSTTTTVGVHLVFDEQGRLITSTPT-----ALVPQA  Fiber12
170 YVTLMGASD----------YVHTLFKHKHVSINVELYFDATGHILPDS  Fiber3
```

FIG. 2 (Continued)

```
     SLGKSYWNFRNGNSIDSTAYTNAVGFMPNLTAYPKPTGSTAK---NNIVG   Majority
                   560       570       580       590       600
472  SLKKHYWNFRNGNSTNANPYTNAVGFMPNLLAYPKTQSQTAK---NNIVS   Fiber2
472  FLDPEYWNFRNGDLTEGTAYTNAVGFMPNLSAYPKSHGKTAK---SNIVS   Fiber5
321  STLKKYWGYKQGDSIDGTPYTNAVGFMPNSTAYPKTQSSTTK---NNIVG   Fiber4
257  NLGKSYWNFRNQNSIMSTAYEKAIGFMPNLVAYPKPTTGSKKYARDIVYG   Fiber8
257  NLGKSYWNFRNENSIMSTAYEKAIGFMPNLVAYPKPTAGSKKYARDIVYG   Fiber9
455  T-----WGYRQGQSAMTH-VSNAVEFWPSSKRYPNEKGSEVQ---NMALT   Fiber40(long)
300  ALESSTWRYQETNAVAS----NALTFMPNSTVYPRNKTADP----GNMLI   Fiber40(short)
489  S-----WGYRQGQSVSTNTVTNGLGFMPNVSAYPRPNASEAK---SQMVS   Fiber12
208  SSLKTDLELKYKQTADF----SARGFMPSTTAYPFVLPHAGTHNENYIFG   Fiber3
```

FIG. 2 (Continued)

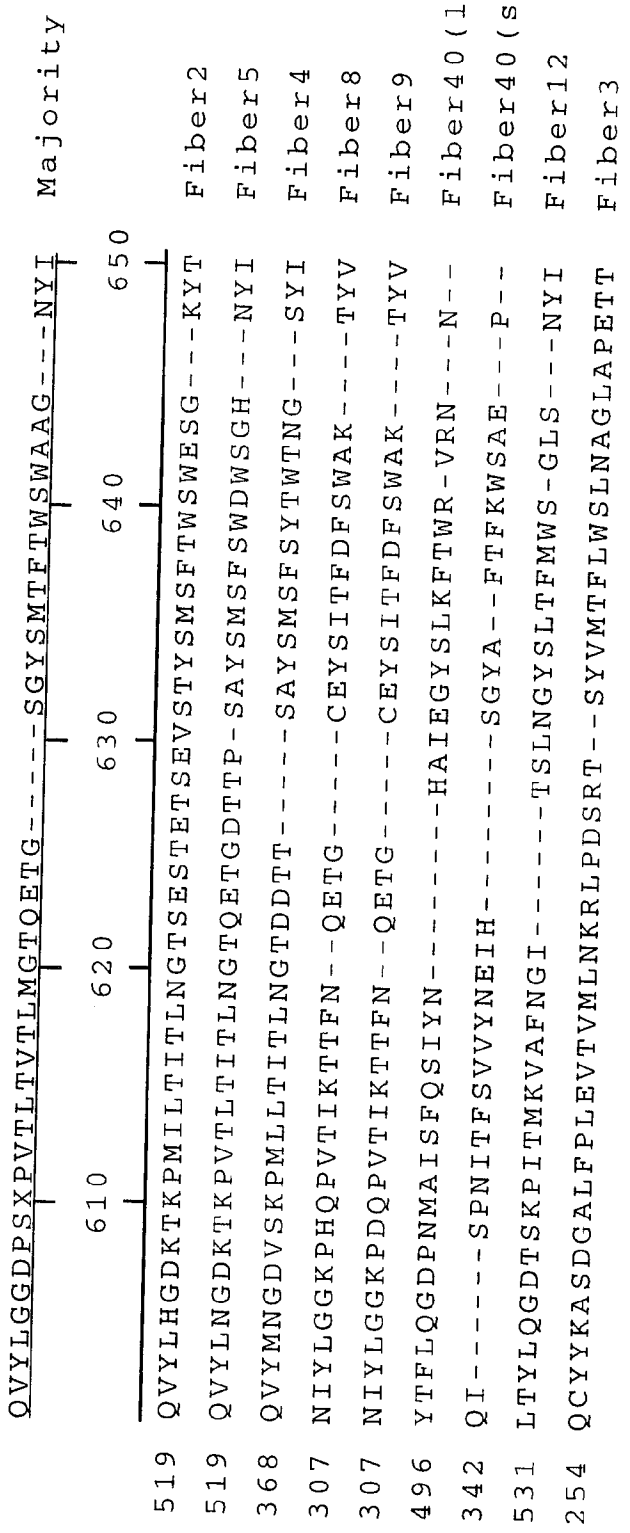


FIG. 2 (Continued)

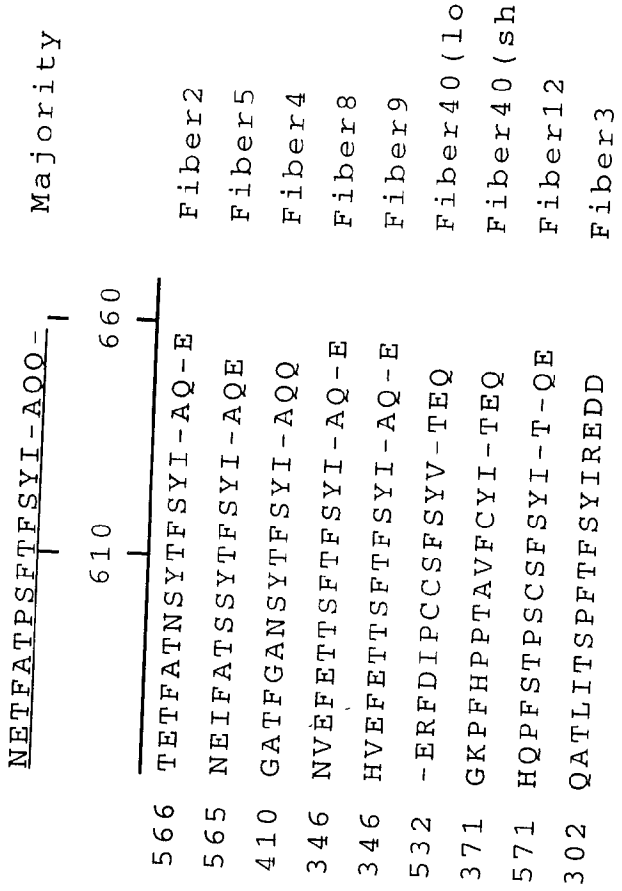


FIG. 2 (Continued)

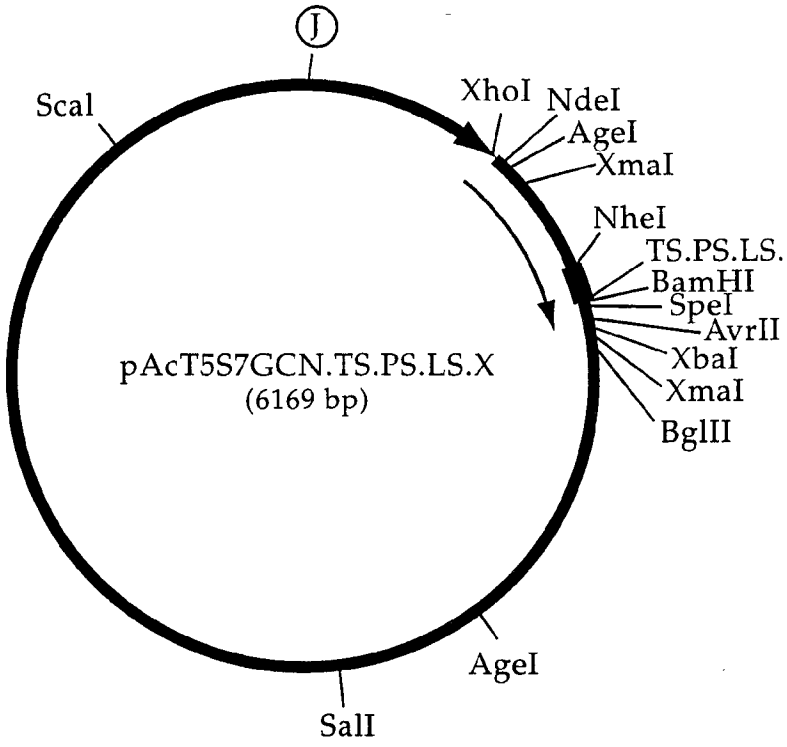

J
ScaI
XhoI
NdeI
AgeI
XmaI
NheI
TS.PS.LS.
BamHI
SpeI
AvrII
XbaI
XmaI
BglII
pAcT5S7GCN.TS.PS.LS.X
(6169 bp)
AgeI
SalI

FIG. 3A

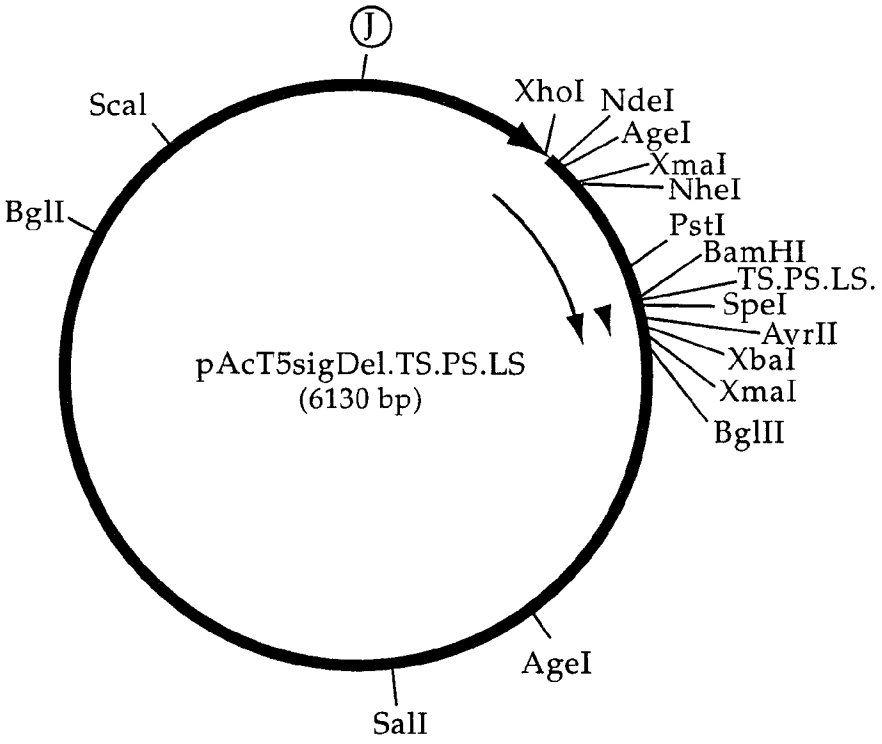

J
ScaI
BglI
XhoI
NdeI
AgeI
XmaI
NheI
PstI
BamHI
TS.PS.LS.
SpeI
AvrII
XbaI
XmaI
BglII
pAcT5sigDel.TS.PS.LS
(6130 bp)
AgeI
SalI

FIG. 3B

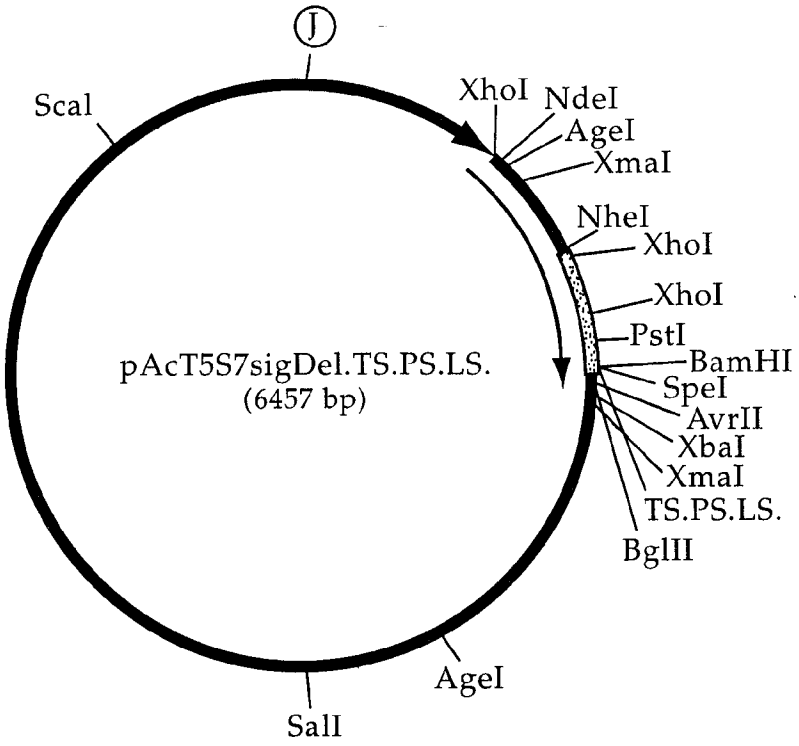

J
ScaI
XhoI
NdeI
AgeI
XmaI
NheI
XhoI
XhoI
PstI
BamHI
SpeI
AvrII
XbaI
XmaI
TS.PS.LS.
BglII
pAcT5S7sigDel.TS.PS.LS.
(6457 bp)
AgeI
SalI

FIG. 3C

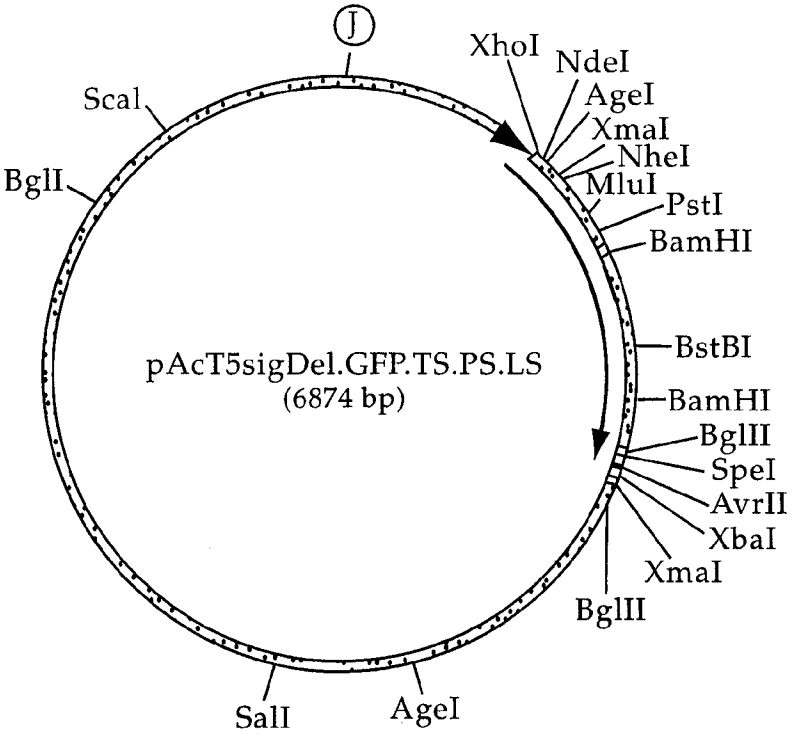

J
XhoI
NdeI
AgeI
XmaI
NheI
MluI
PstI
BamHI
ScaI
BglI
BstBI
BamHI
BglII
SpeI
AvrII
XbaI
XmaI
BglII
pAcT5sigDel.GFP.TS.PS.LS
(6874 bp)
SalI
AgeI

FIG. 4

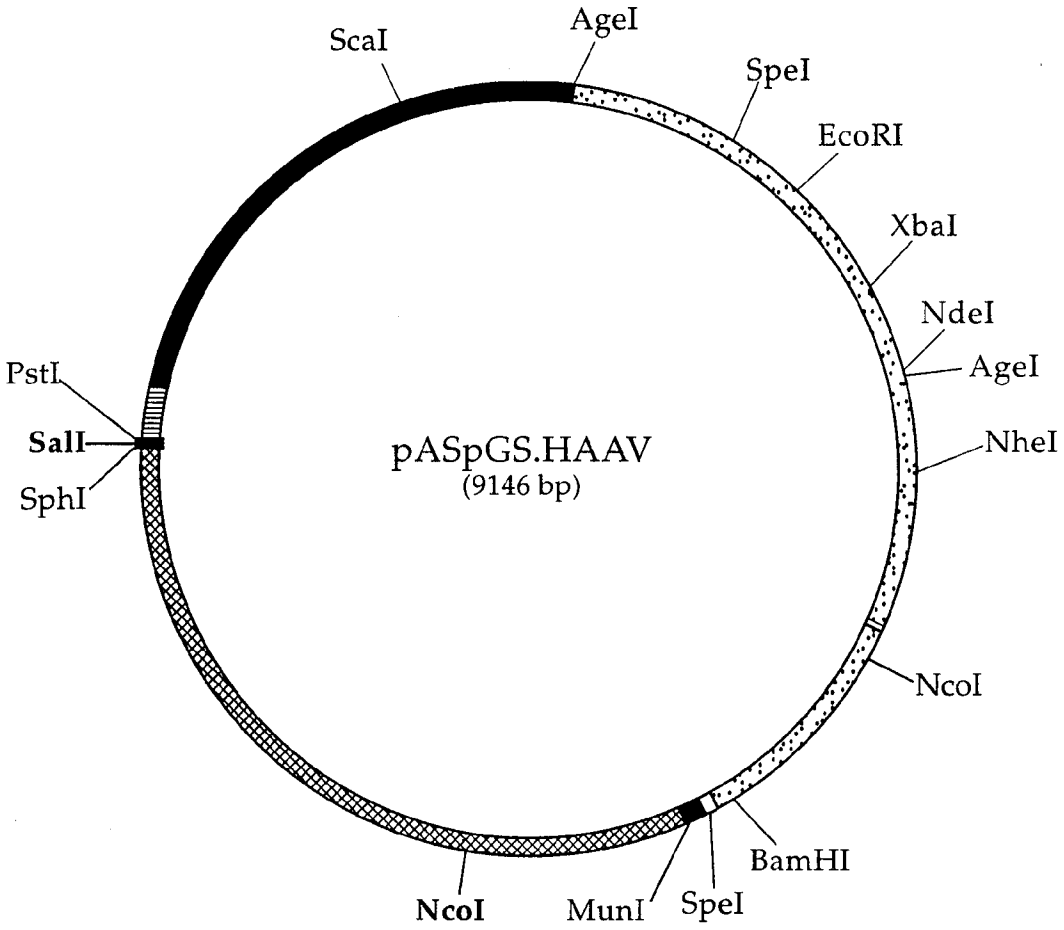

ScaI
AgeI
SpeI
EcoRI
XbaI
NdeI
AgeI
NheI
NcoI
BamHI
SpeI
MunI
NcoI
SphI
SalI
PstI
pASpGS.HAAV
(9146 bp)

FIG. 5A

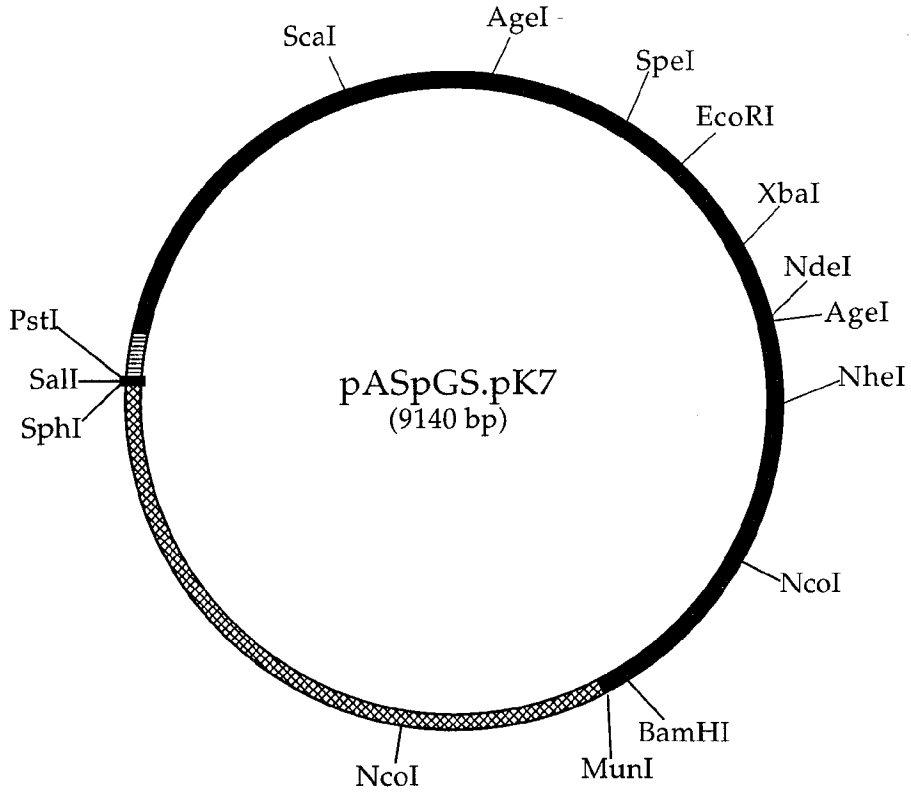

ScaI
AgeI
SpeI
EcoRI
XbaI
NdeI
AgeI
NheI
NcoI
BamHI
MunI
NcoI
PstI
SalI
SphI
pASpGS.pK7
(9140 bp)

FIG. 5B

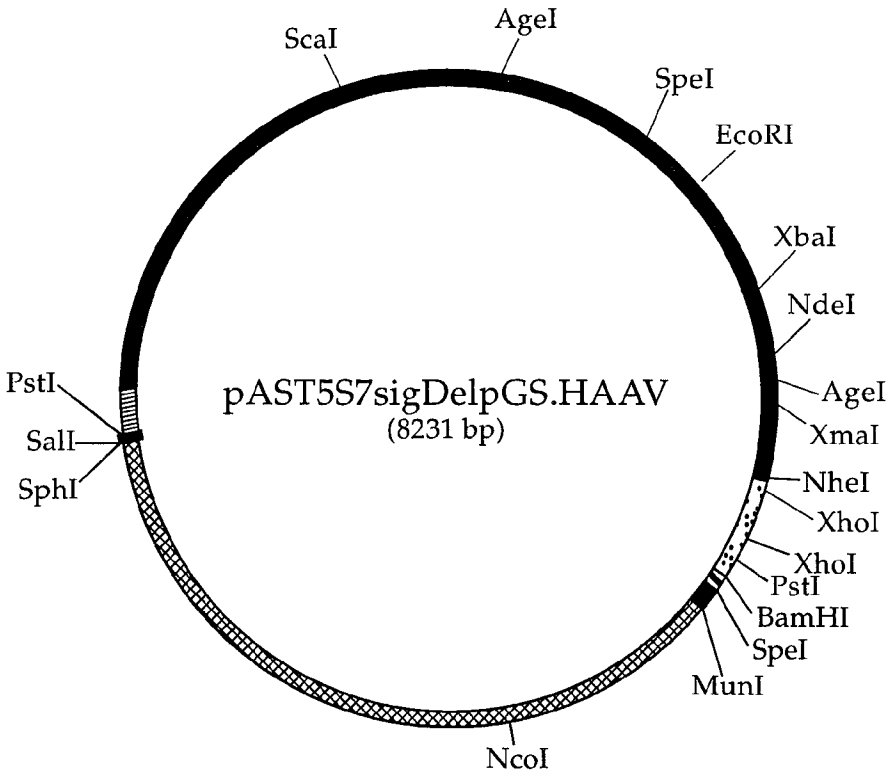

ScaI
AgeI
SpeI
EcoRI
XbaI
NdeI
AgeI
XmaI
NheI
XhoI
XhoI
PstI
BamHI
SpeI
MunI
NcoI
PstI
SalI
SphI
pAST5S7sigDelpGS.HAAV
(8231 bp)

FIG. 5C

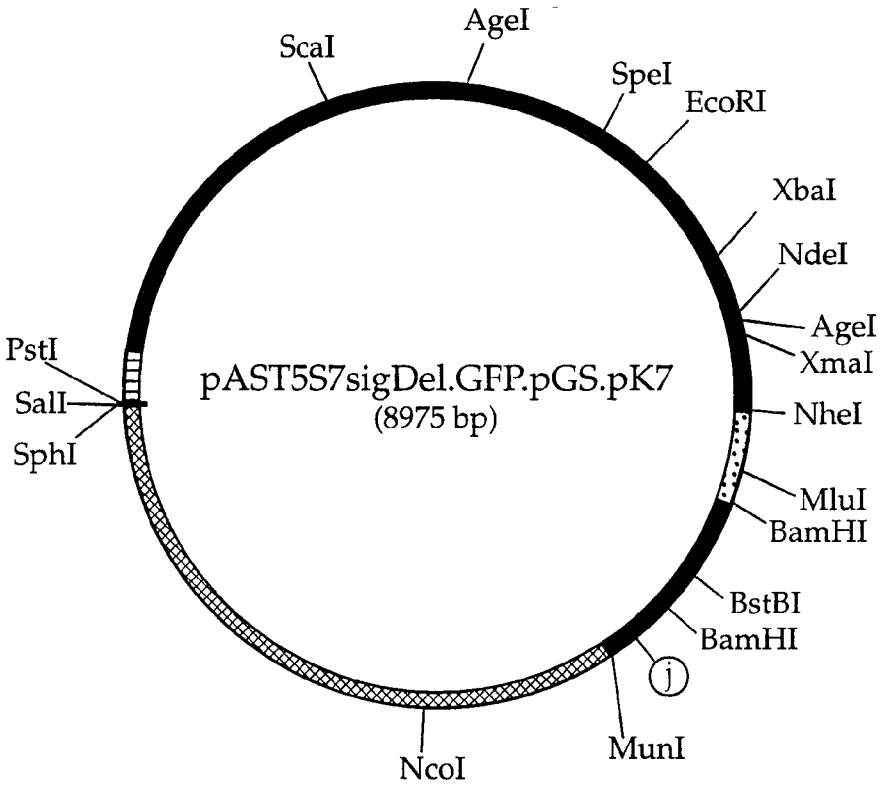
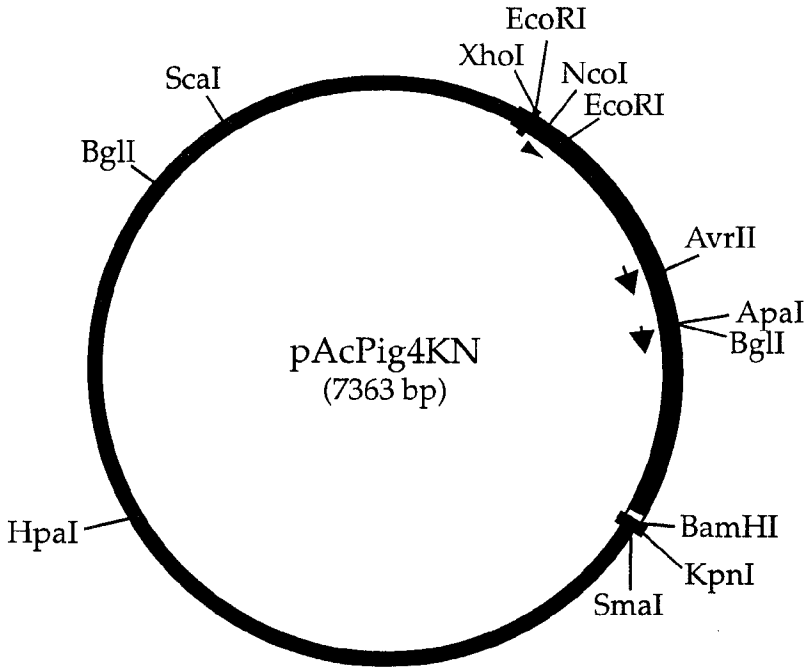

AgeI
ScaI
SpeI
EcoRI
XbaI
NdeI
AgeI
XmaI
NheI
MluI
BamHI
BstBI
BamHI
j
MunI
NcoI
PstI
SalI
SphI
pAST5S7sigDel.GFP.pGS.pK7
(8975 bp)

FIG. 5D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XhoI
NcoI
EcoRI
ScaI
BglI
AvrII
ApaI
BglI
HpaI
BamHI
KpnI
SmaI
pAcPig4KN
(7363 bp)

FIG. 6A

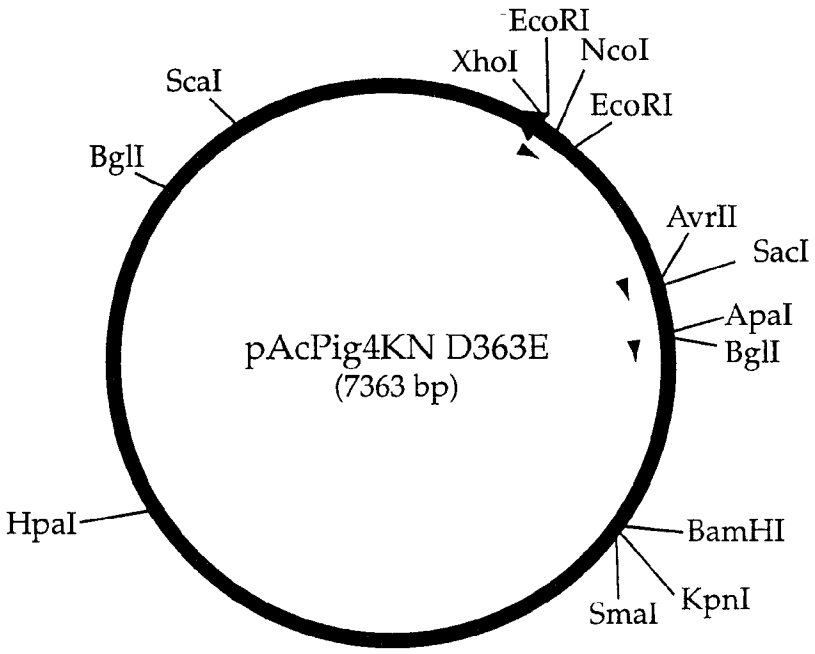

EcoRI
XhoI
NcoI
EcoRI
ScaI
BglI
AvrII
SacI
ApaI
BglI
pAcPig4KN D363E
(7363 bp)
HpaI
BamHI
KpnI
SmaI

FIG. 6B

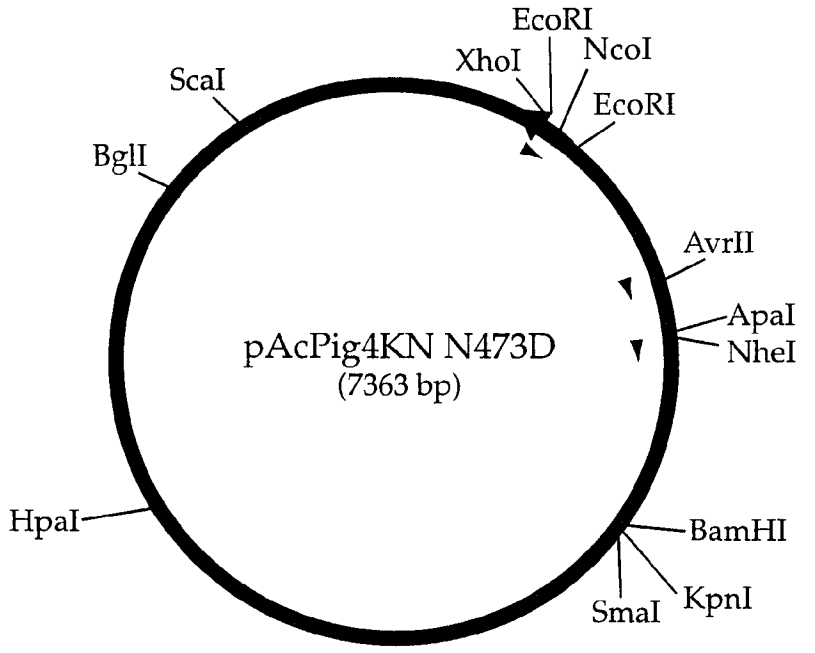
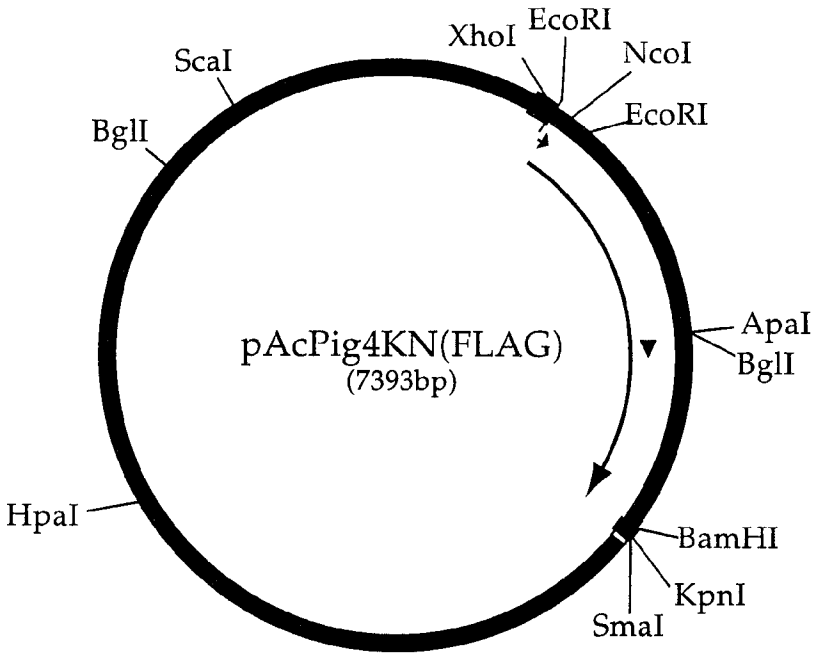

EcoRI
XhoI
NcoI
ScaI
EcoRI
BglI
AvrII
ApaI
NheI
pAcPig4KN N473D
(7363 bp)
HpaI
BamHI
KpnI
SmaI

FIG. 6C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EcoRI
NcoI
EcoRI
ScaI
BglI
pAcPig4KN(FLAG)
(7393bp)
ApaI
BglI
HpaI
BamHI
KpnI
SmaI

FIG. 6D

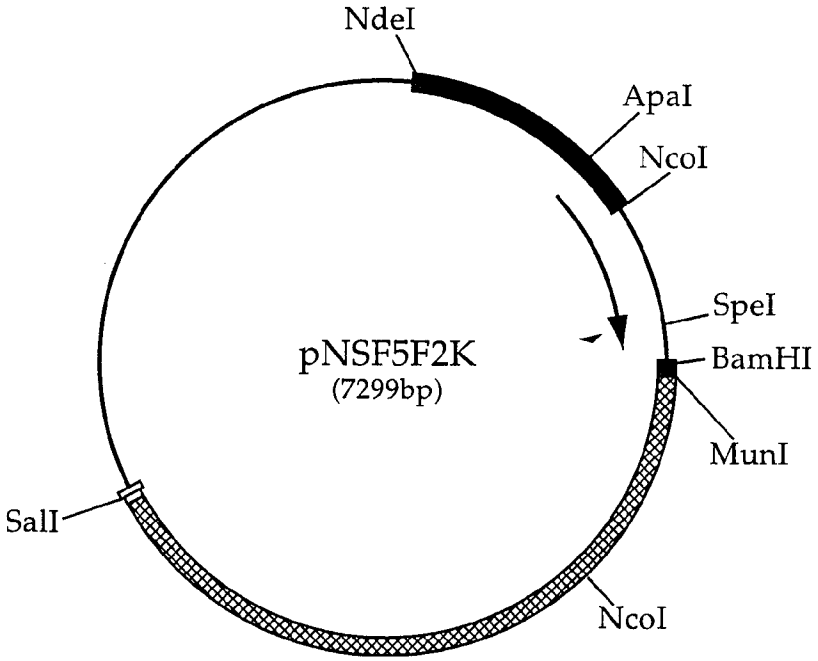
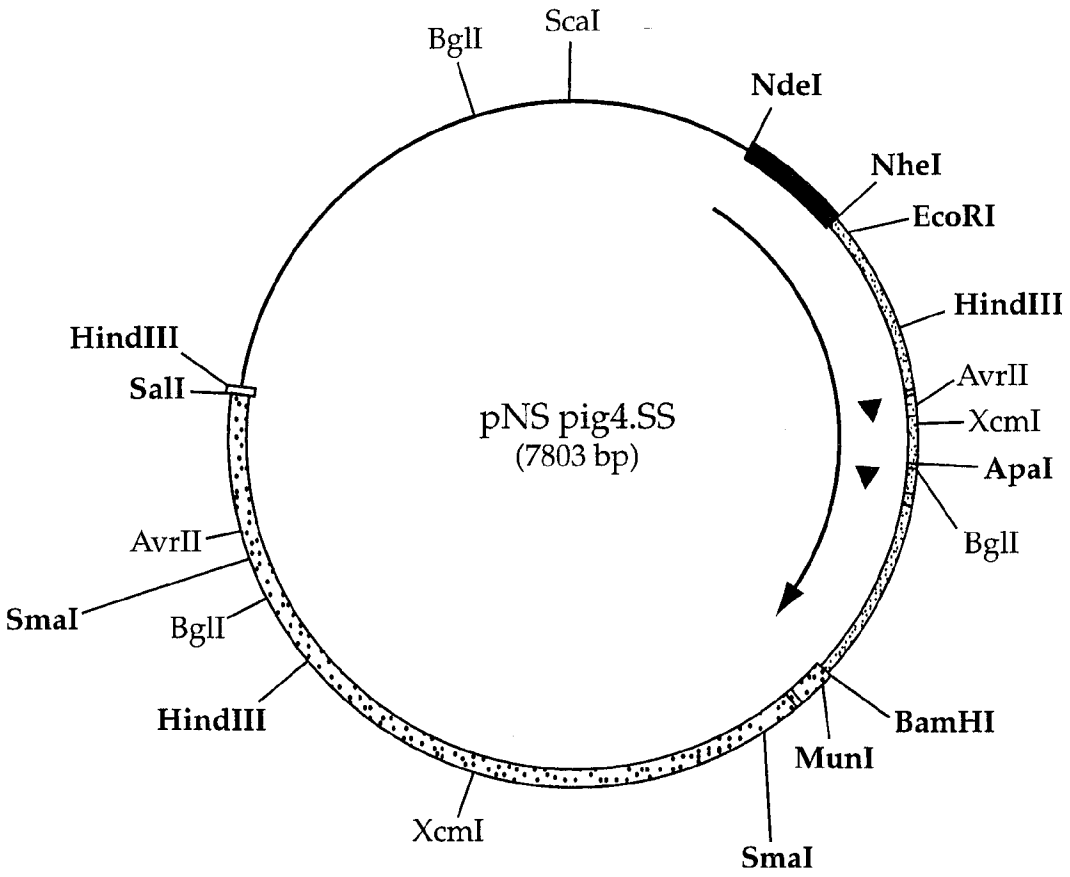

NdeI
ApaI
NcoI
SpeI
BamHI
pNSF5F2K
(7299bp)
MunI
SalI
NcoI

FIG. 7A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ScaI
NdeI
NheI
EcoRI
HindIII
AvrII
XcmI
ApaI
BglI
BamHI
MunI
SmaI
XcmI
HindIII
BglI
AvrII
SmaI
HindIII
SalI
pNS pig4.SS
(7803 bp)

FIG. 7B

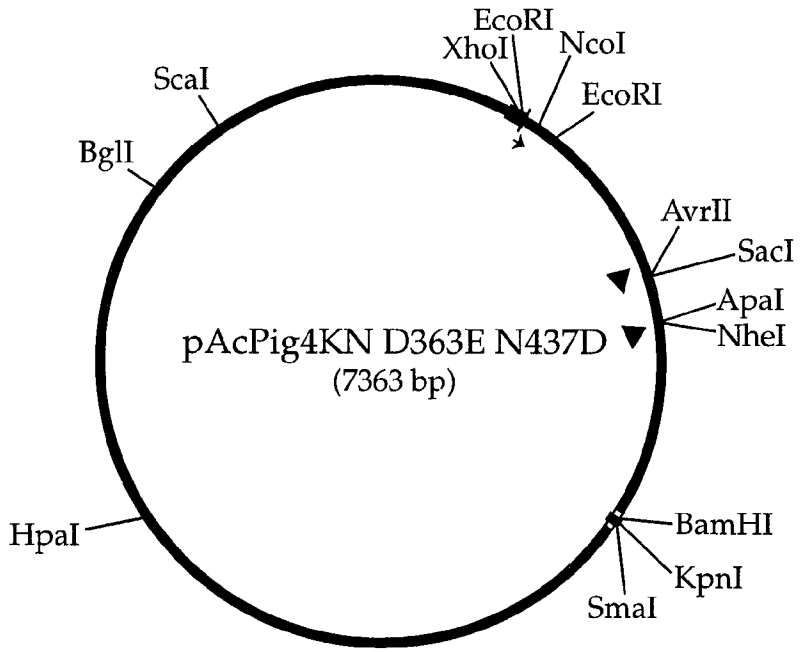
EcoRI
XhoI
NcoI
EcoRI
ScaI
BglI
AvrII
SacI
ApaI
NheI
pAcPig4KN D363E N437D
(7363 bp)
BamHI
KpnI
SmaI
HpaI

FIG. 8A

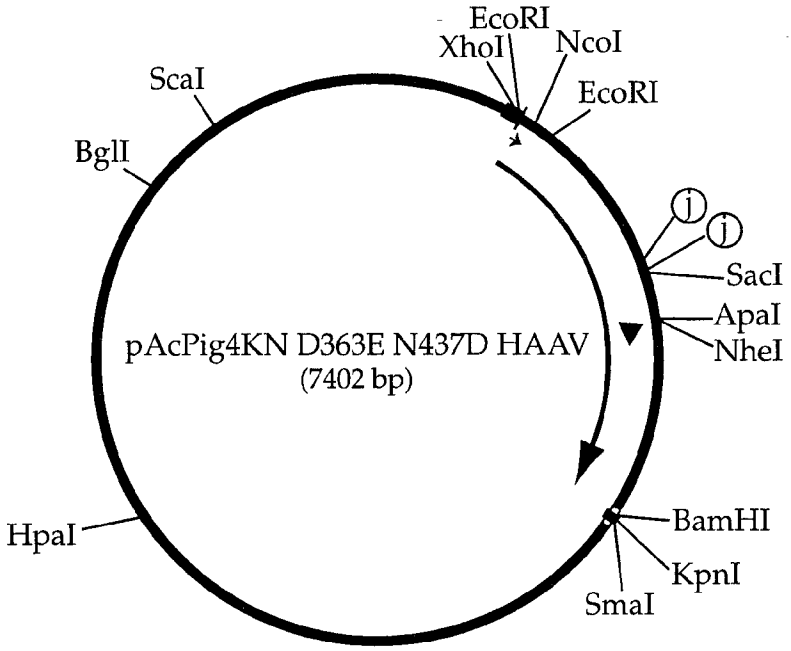
EcoRI
XhoI
NcoI
EcoRI
ScaI
BglI
SacI
ApaI
NheI
pAcPig4KN D363E N437D HAAV
(7402 bp)
HpaI
BamHI
KpnI
SmaI

FIG. 8B

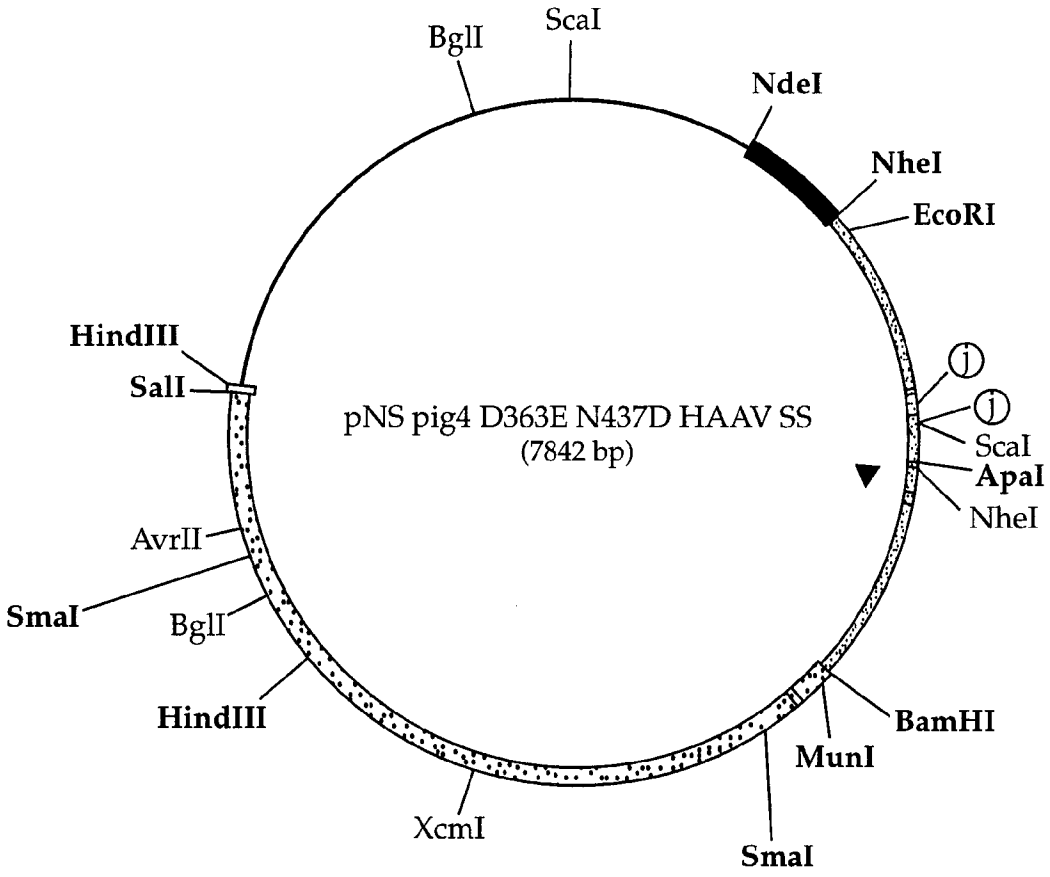
BglI
ScaI
NdeI
NheI
EcoRI
HindIII
SalI
ScaI
ApaI
NheI
pNS pig4 D363E N437D HAAV SS
(7842 bp)
AvrII
SmaI
BglI
HindIII
BamHI
MunI
XcmI
SmaI

FIG. 8C

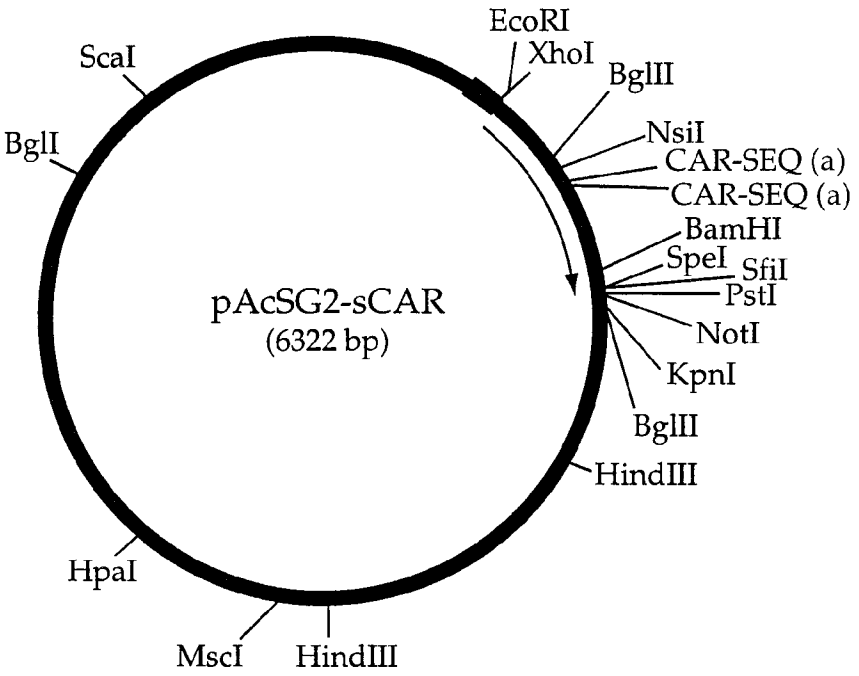
EcoRI
XhoI
BglII
NsiI
CAR-SEQ (a)
CAR-SEQ (a)
BamHI
SpeI
SfiI
PstI
NotI
KpnI
BglII
HindIII
HpaI
MscI
HindIII
ScaI
BglI
pAcSG2-sCAR
(6322 bp)

FIG. 9A

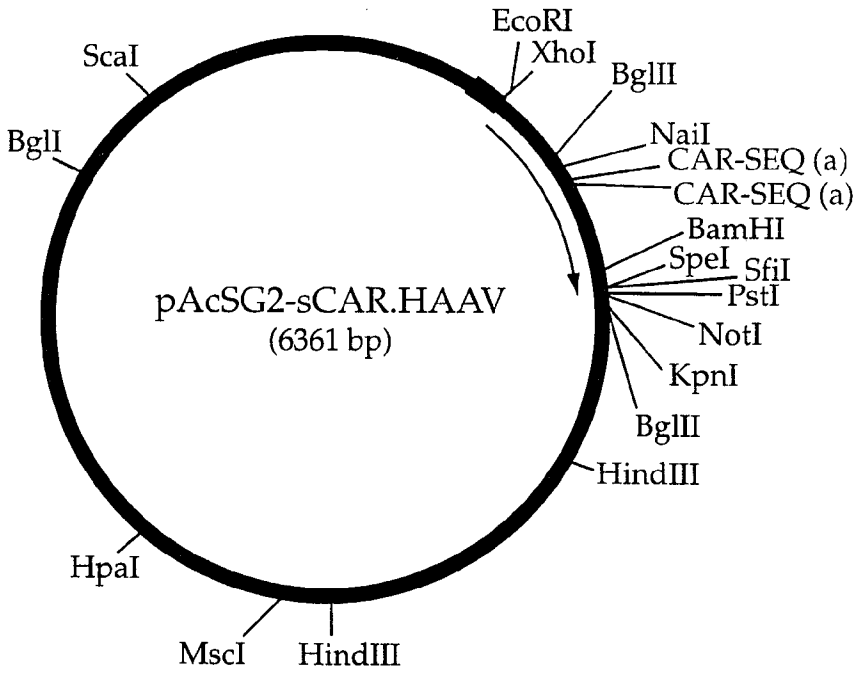
EcoRI
XhoI
BglII
NaiI
CAR-SEQ (a)
CAR-SEQ (a)
BamHI
SpeI
SfiI
PstI
NotI
KpnI
BglII
HindIII
HpaI
MscI
HindIII
ScaI
BglI
pAcSG2-sCAR.HAAV
(6361 bp)

FIG. 9B

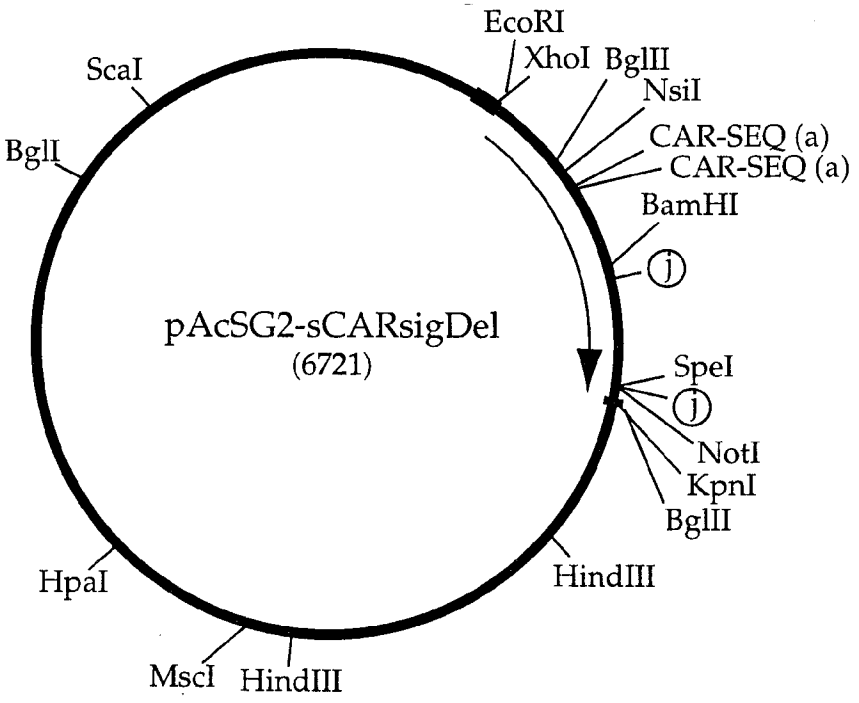

FIG. 10A

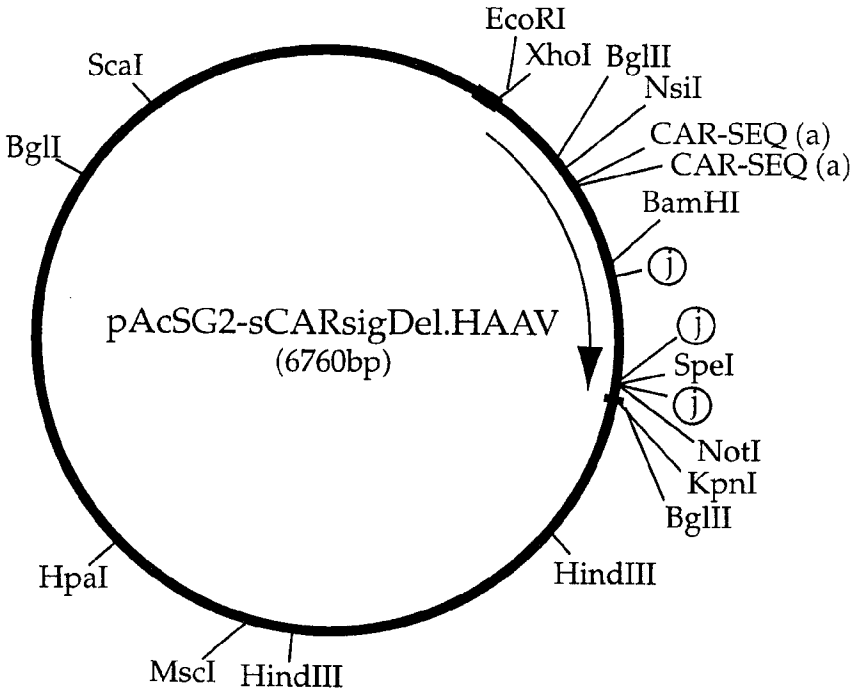

FIG. 10B

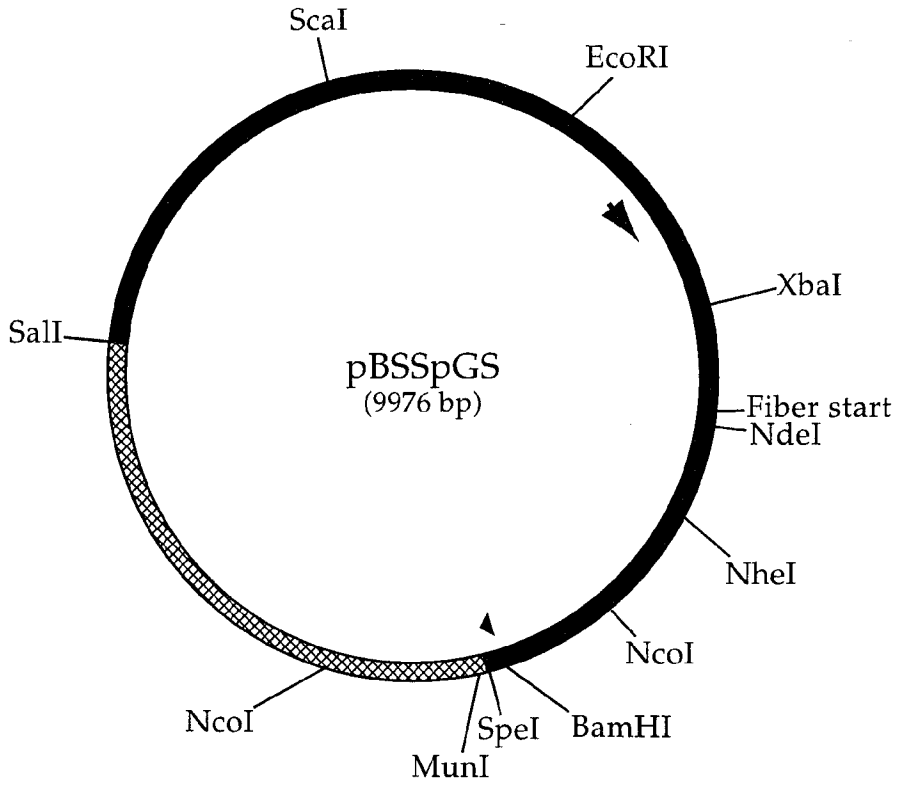

FIG. 11A

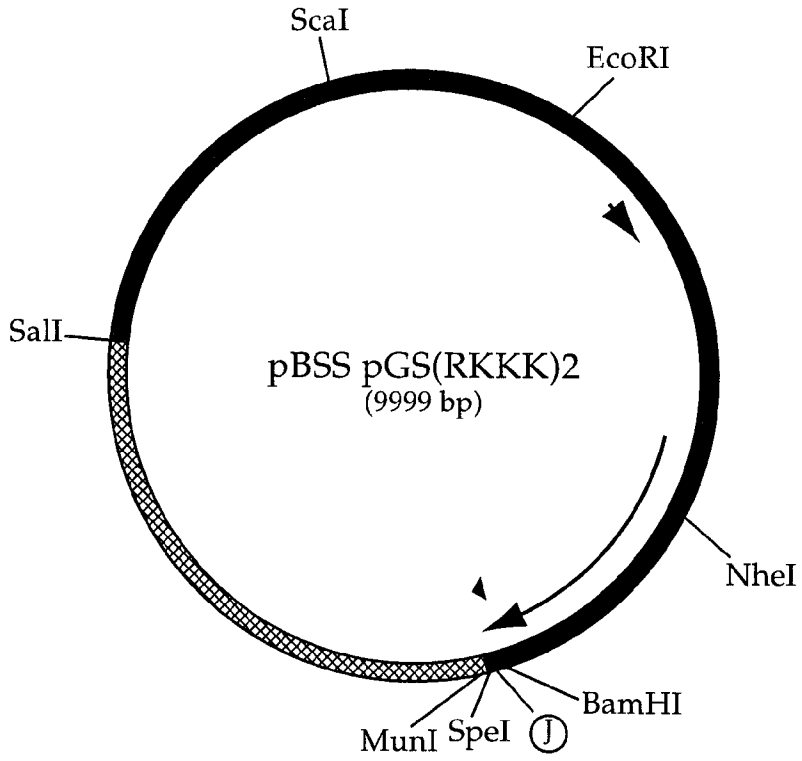

FIG. 11B

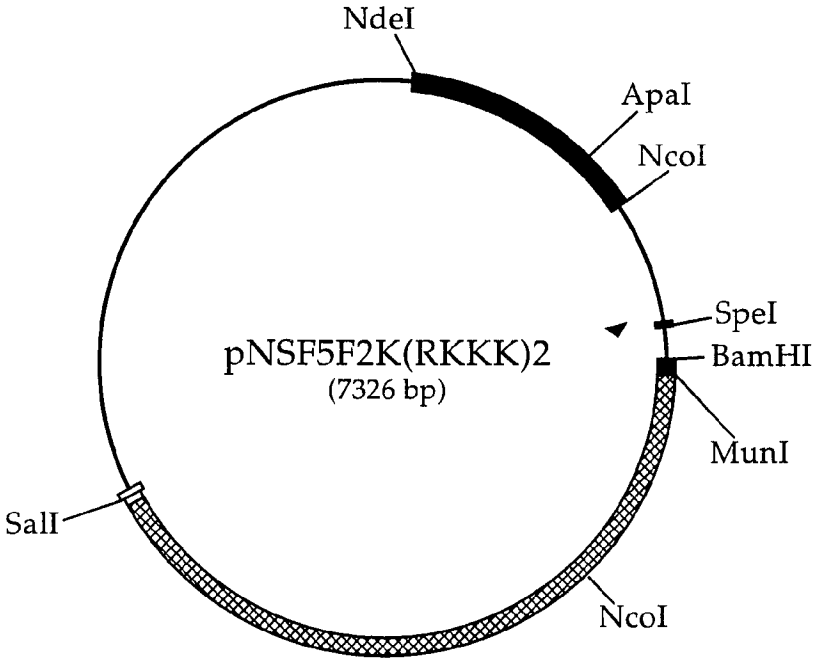

FIG. 11C

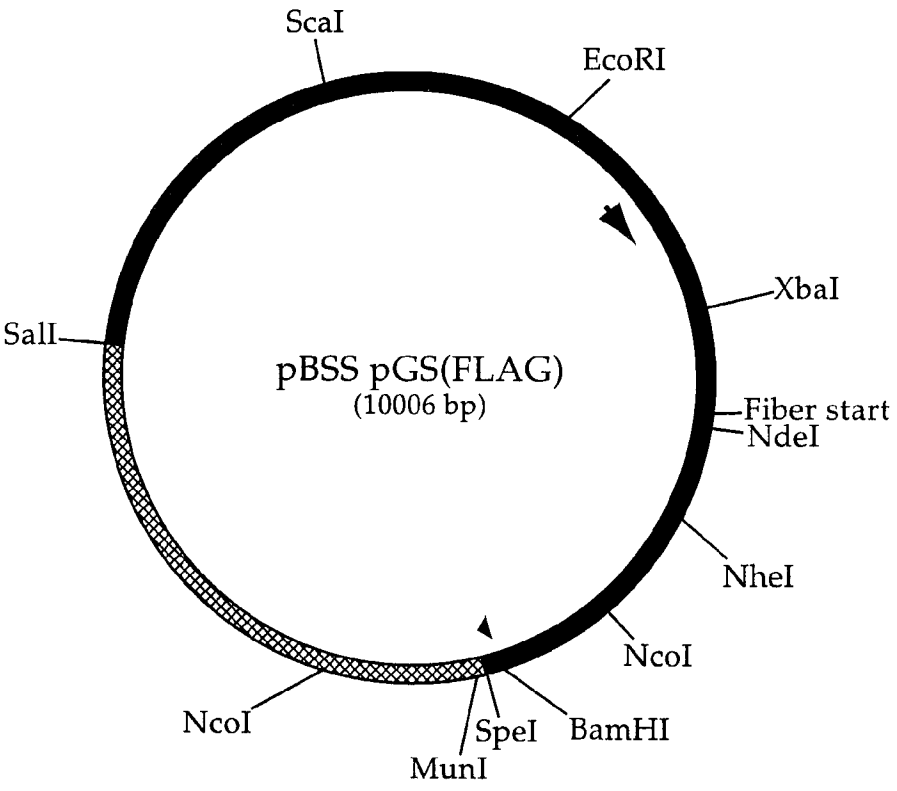

FIG. 11D

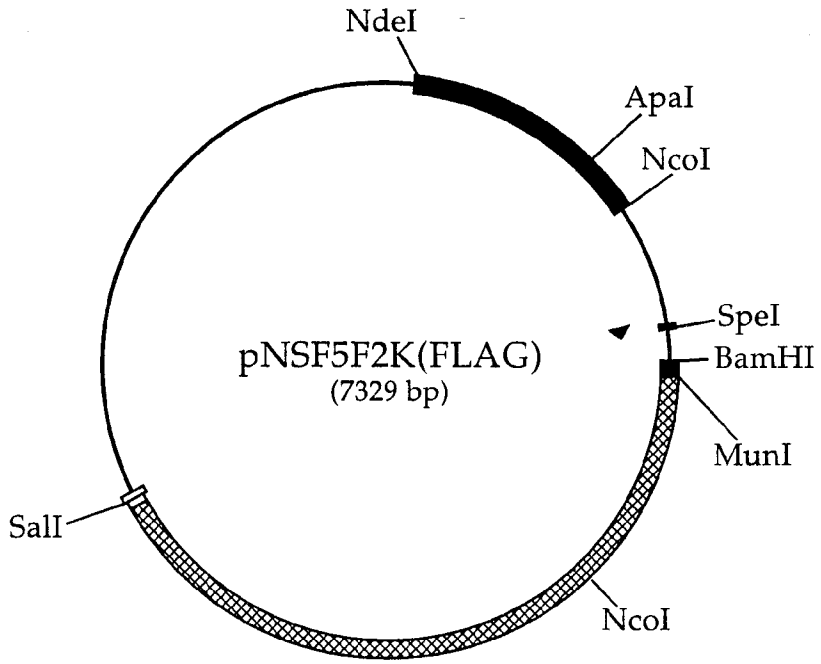

FIG. 11E

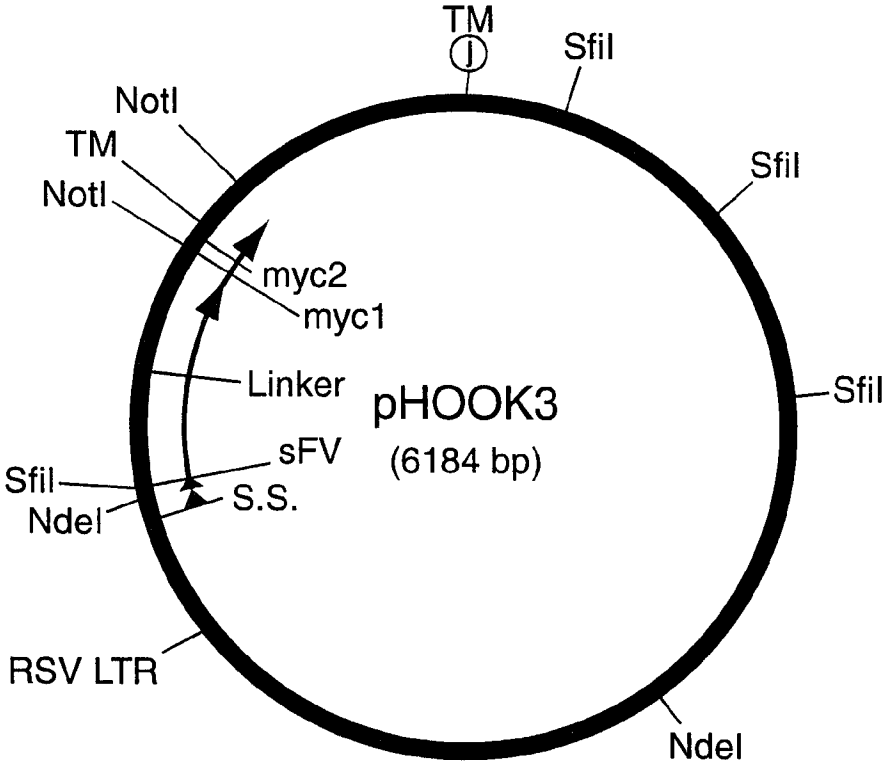

FIG. 12A

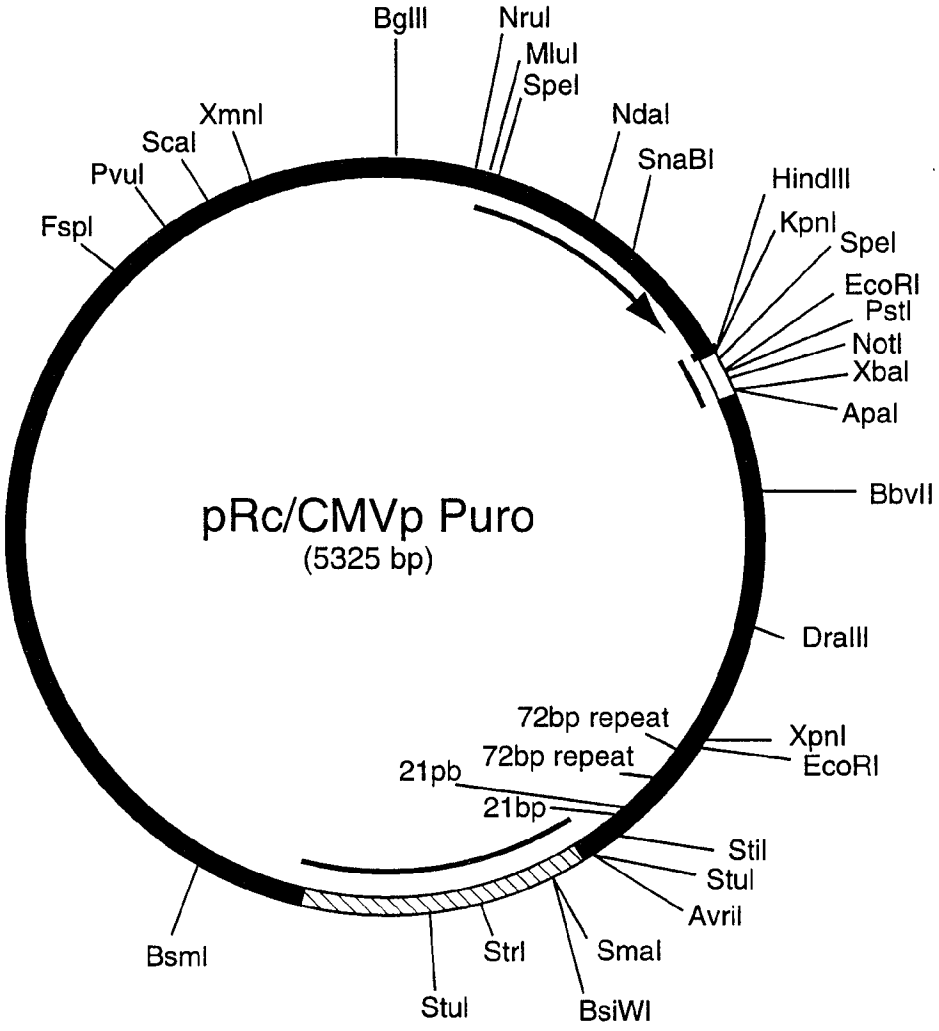
BglII
NruI
MluI
SpeI
NdaI
SnaBI
HindIII
KpnI
SpeI
EcoRI
PstI
NotI
XbaI
ApaI
BbvII
DraIII
XpnI
EcoRI
StiI
StuI
AvrII
SmaI
BsiWI
StuI
StrI
BsmI
FspI
PvuI
ScaI
XmnI
pRc/CMVp Puro
(5325 bp)
72bp repeat
72bp repeat
21pb
21bp

FIG. 12B

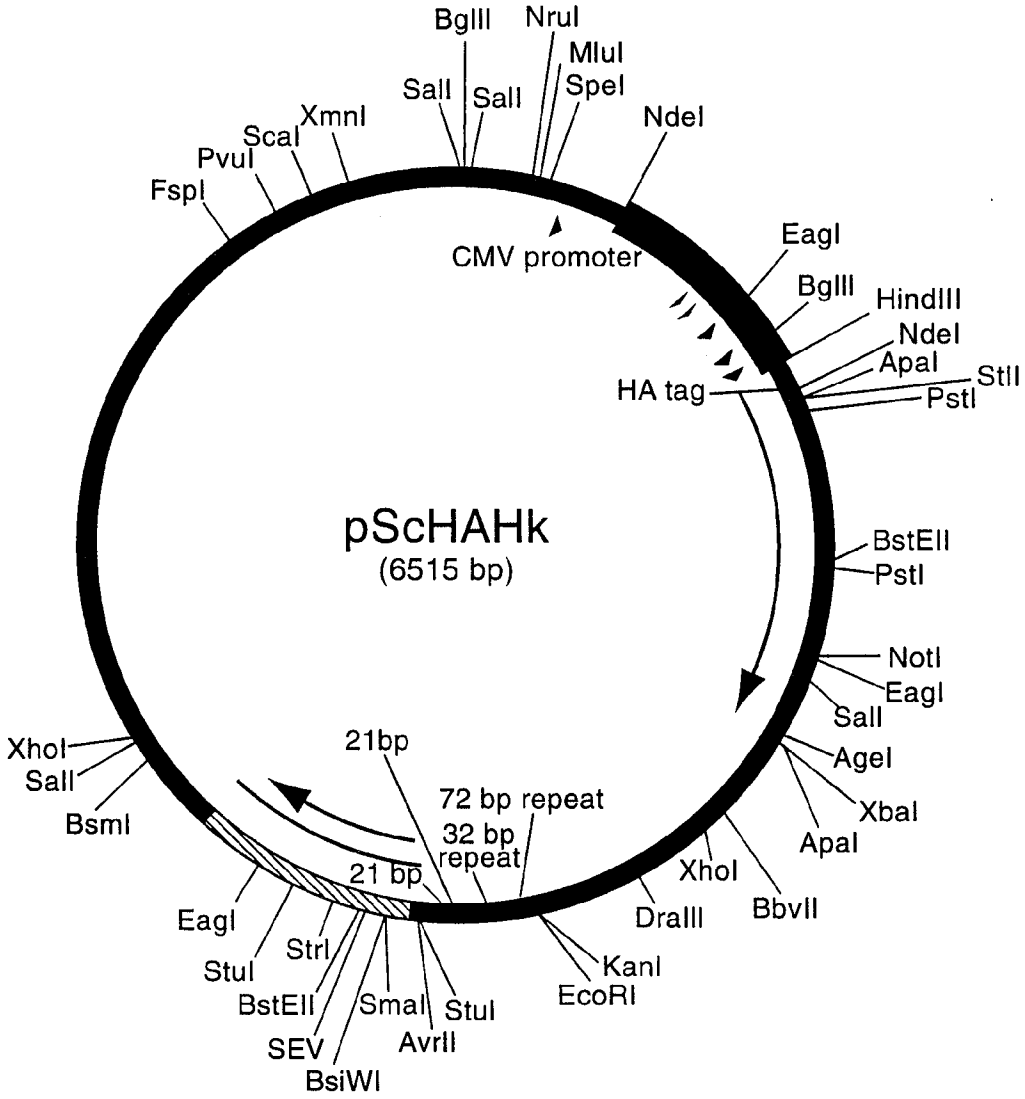
BglII
NruI
MluI
SpeI
SalI
SalI
NdeI
XmnI
ScaI
PvuI
FspI
CMV promoter
EagI
BglII
HindIII
NdeI
ApaI
StuI
PstI
HA tag
pScHAHk
(6515 bp)
BstEII
PstI
NotI
EagI
SalI
AgeI
XbaI
ApaI
XhoI
BbvII
DraIII
KanI
EcoRI
StuI
AvrII
SmaI
BstEII
StrI
StuI
EagI
21 bp
32 bp
repeat
72 bp repeat
21bp
BsmI
SalI
XhoI
SEV
BsiWI

FIG 12C

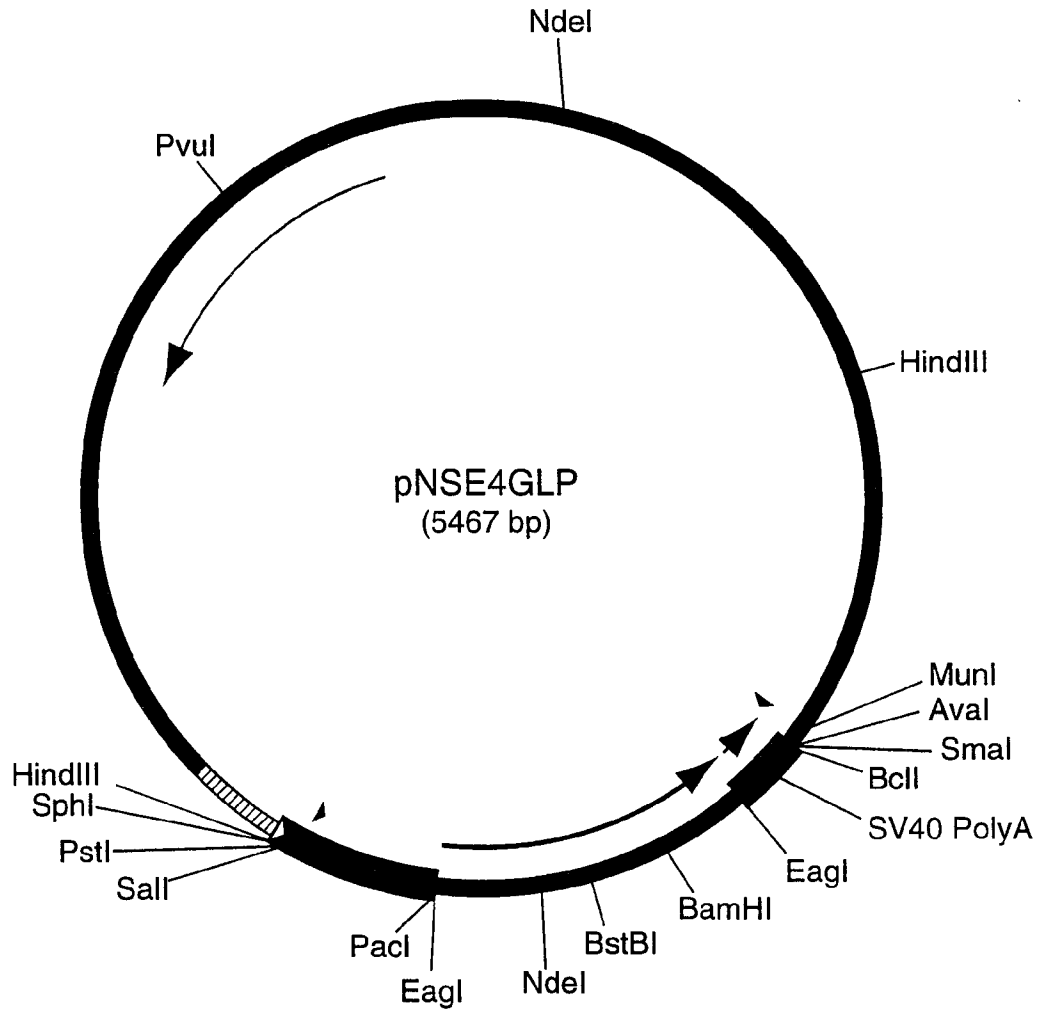
NdeI
PvuI
HindIII
pNSE4GLP
(5467 bp)
MunI
AvaI
SmaI
BclI
SV40 PolyA
EagI
BamHI
BstBI
NdeI
EagI
PacI
SalI
PstI
SphI
HindIII

FIG. 12D

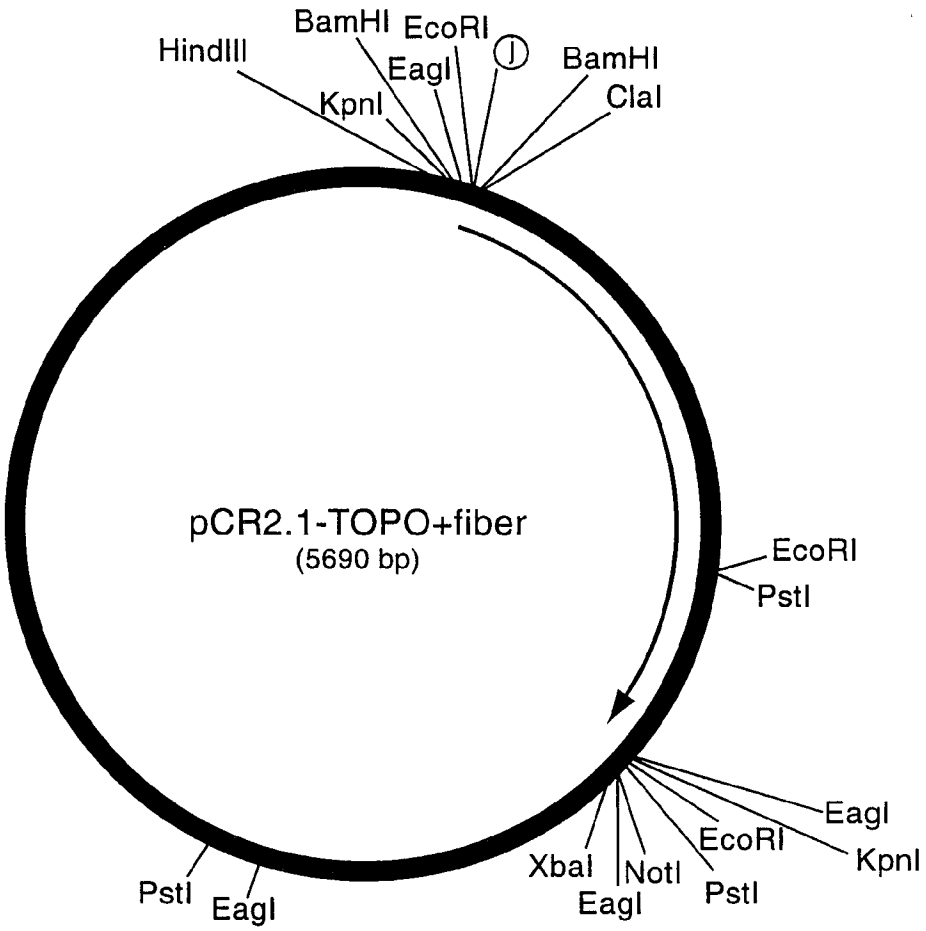
HindIII
BamHI
EcoRI
J
BamHI
EagI
ClaI
KpnI
pCR2.1-TOPO+fiber
(5690 bp)
EcoRI
PstI
EagI
KpnI
EcoRI
XbaI
NotI
PstI
EagI
PstI
EagI

FIG. 13A

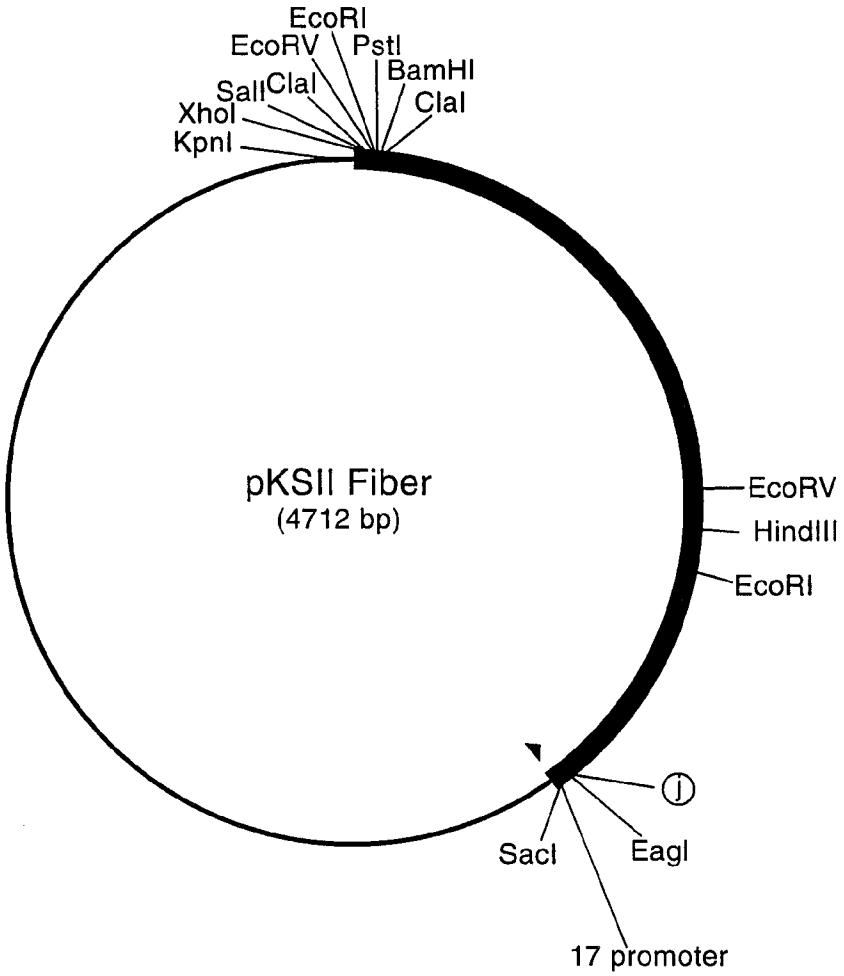
EcoRI
EcoRV
PstI
BamHI
SalI
ClaI
XhoI
ClaI
KpnI
pKSII Fiber
(4712 bp)
EcoRV
HindIII
EcoRI
J
SacI
EagI
17 promoter

FIG. 13B

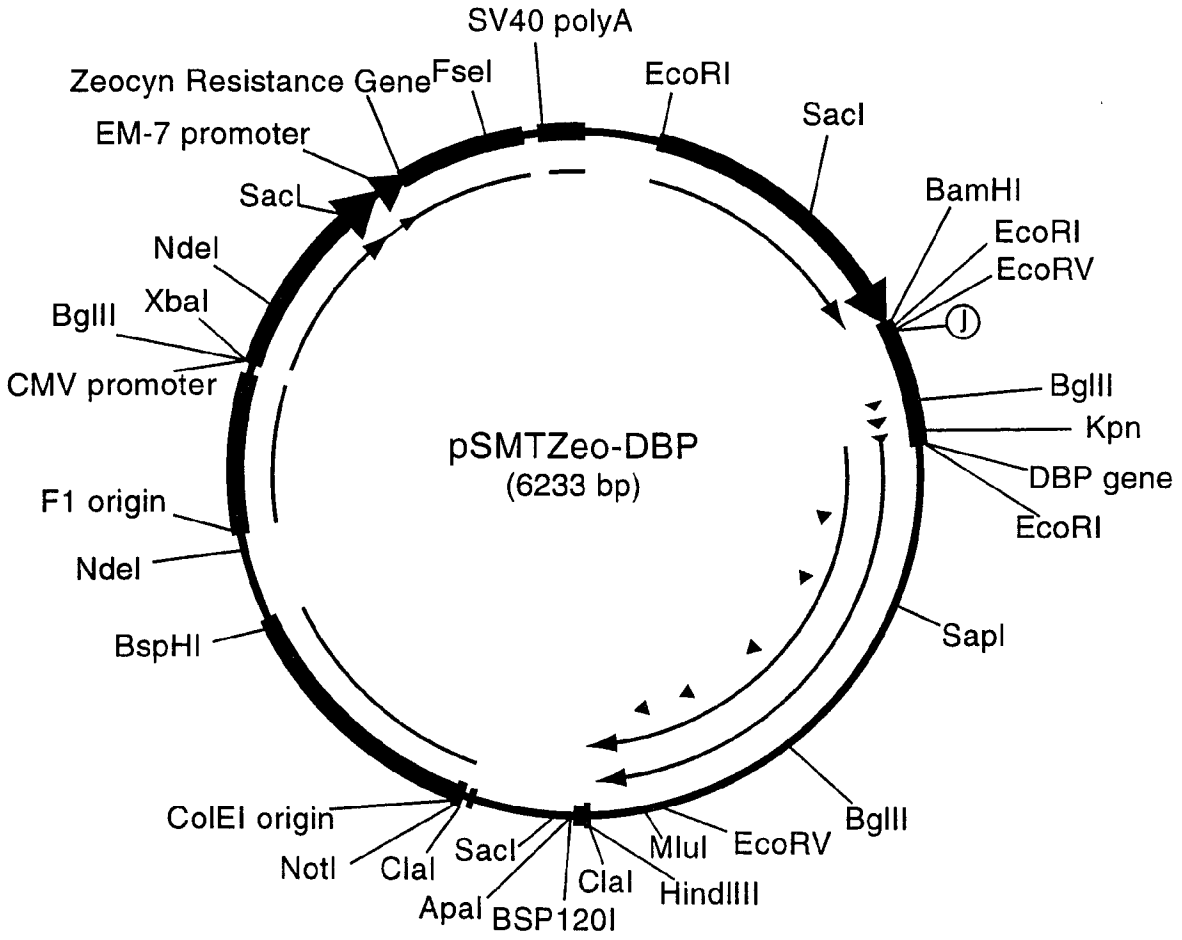
SV40 polyA
Zeocyn Resistance Gene
FseI
EcoRI
SacI
EM-7 promoter
SacI
BamHI
EcoRI
EcoRV
NdeI
XbaI
BglII
CMV promoter
pSMTZeo-DBP
(6233 bp)
BglII
KpnI
DBP gene
EcoRI
F1 origin
NdeI
BspHI
SapI
ColEI origin
NotI
ClaI
SacI
ApaI
BSP120I
ClaI
MluI
EcoRV
HindIII
BglII

FIG. 13C

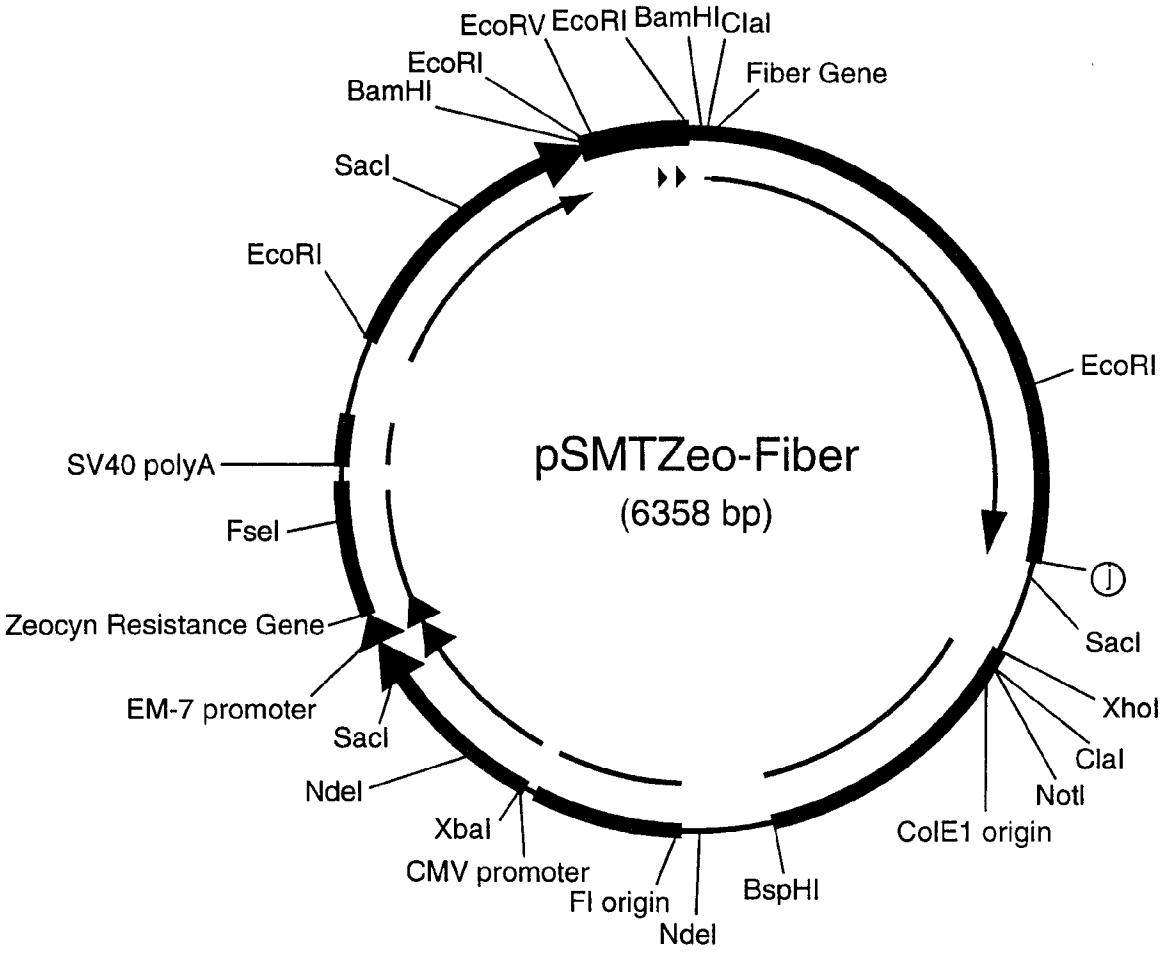
EcoRV
EcoRI
BamHI
ClaI
Fiber Gene
EcoRI
BamHI
SacI
EcoRI
EcoRI
pSMTZeo-Fiber
(6358 bp)
SV40 polyA
FseI
Zeocyn Resistance Gene
EM-7 promoter
SacI
NdeI
XbaI
CMV promoter
F1 origin
NdeI
BspHI
ColE1 origin
NotI
ClaI
XhoI
SacI

FIG. 13D

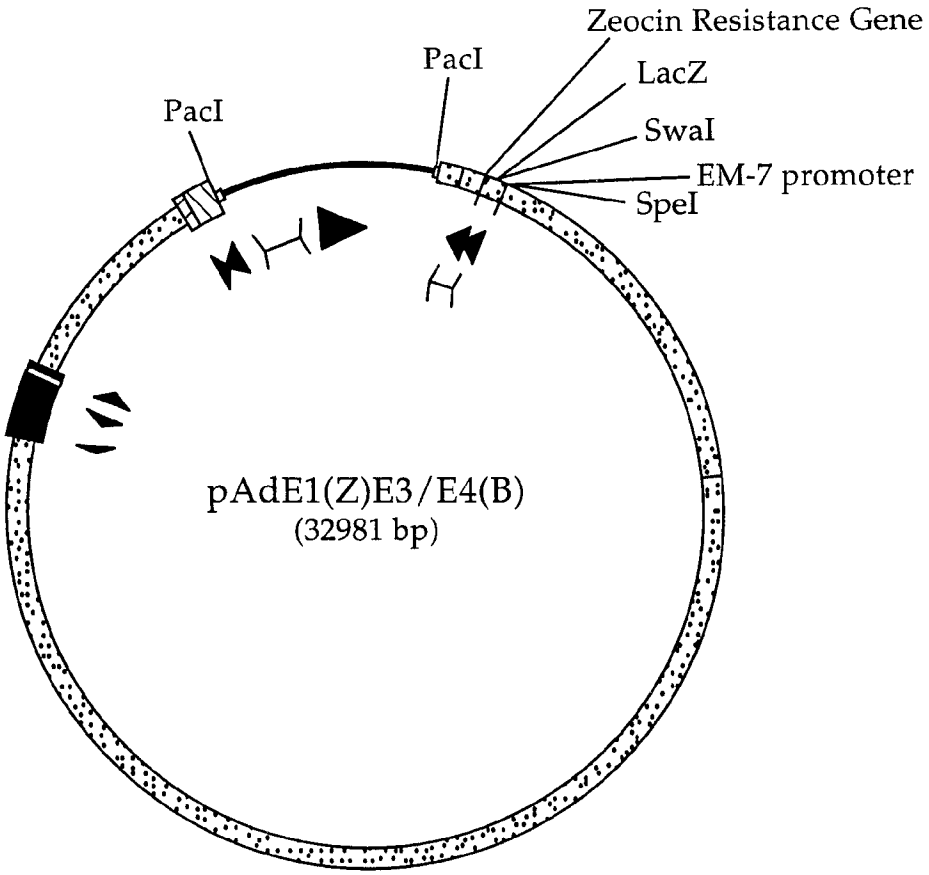
Zeocin Resistance Gene
PacI
LacZ
PacI
SwaI
EM-7 promoter
SpeI
pAdE1(Z)E3/E4(B)
(32981 bp)

FIG. 14

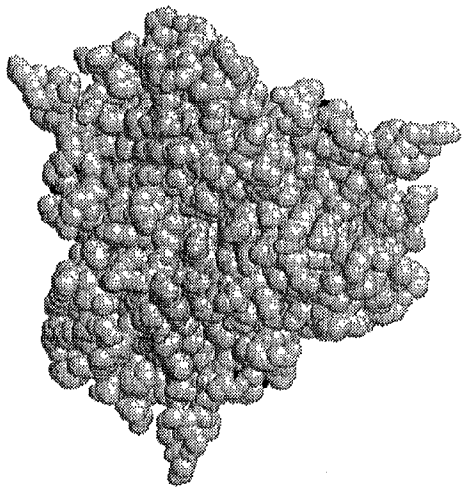

FIG. 15A

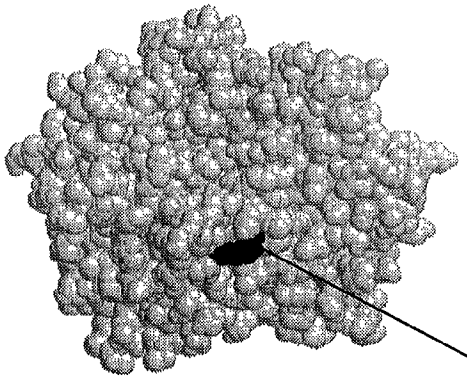

FIG. 15B

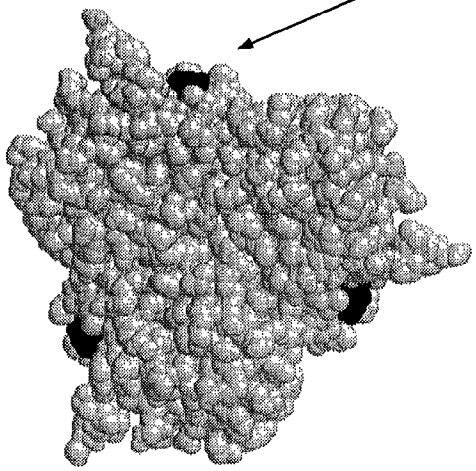

FIG. 15C

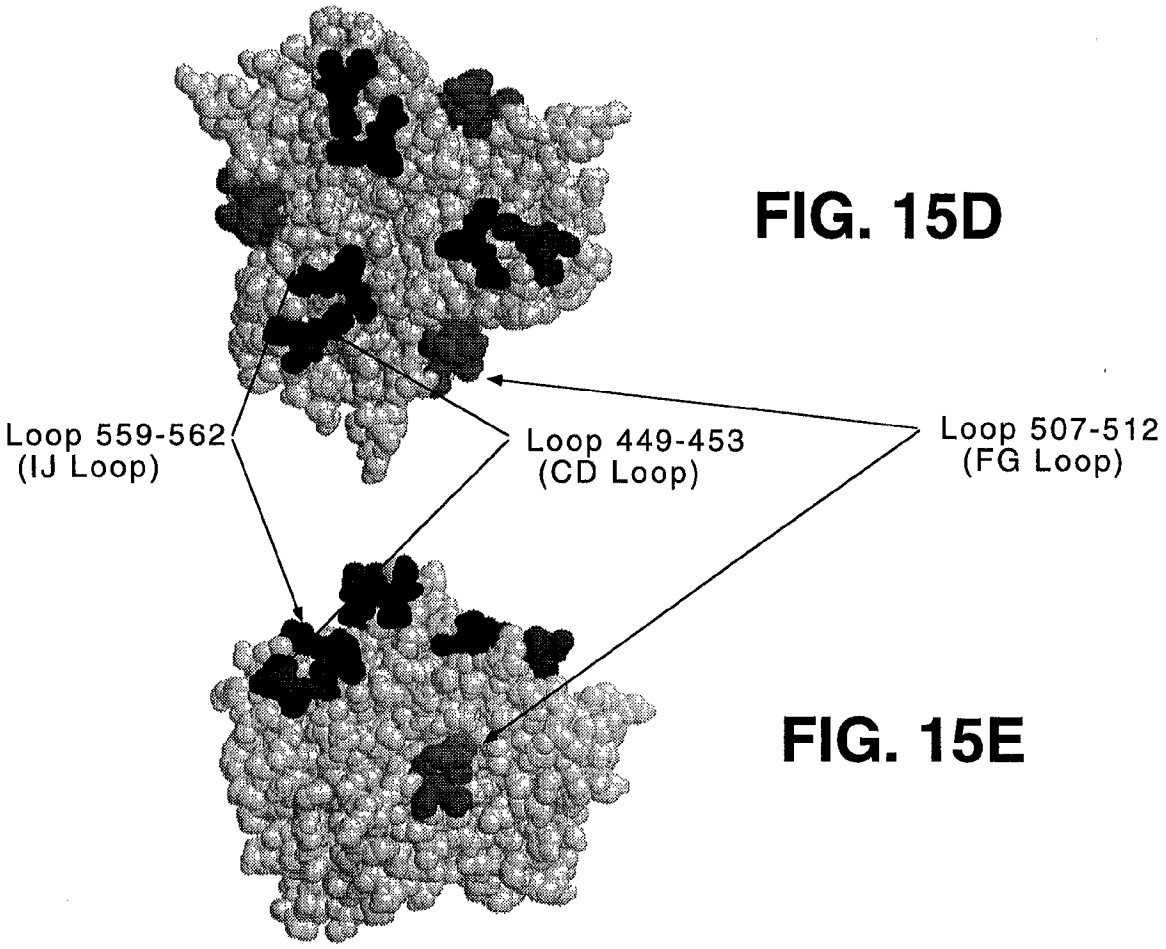

FIG. 15D

FIG. 15E

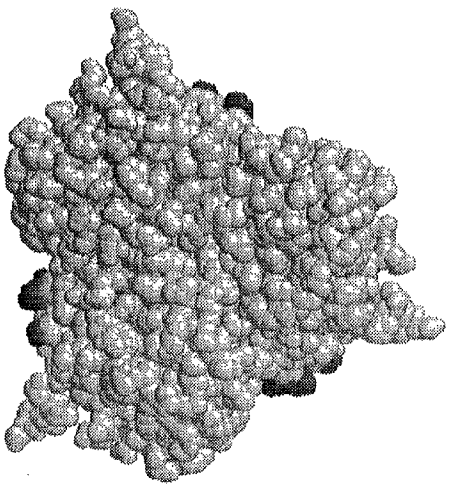

FIG. 15F

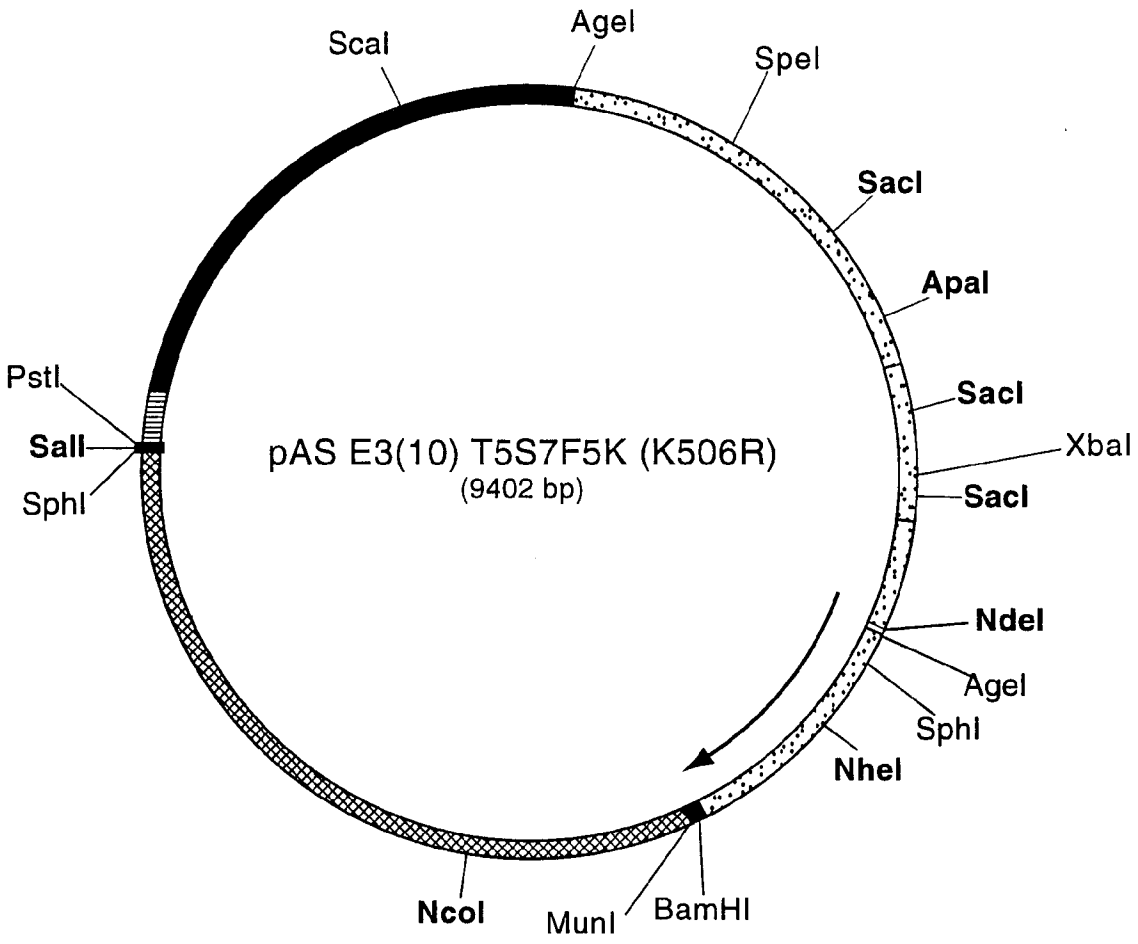
ScaI
AgeI
SpeI
SacI
ApaI
SacI
XbaI
SacI
NdeI
AgeI
SphI
NheI
BamHI
MunI
NcoI
PstI
SalI
SphI
pAS E3(10) T5S7F5K (K506R)
(9402 bp)

FIG. 16

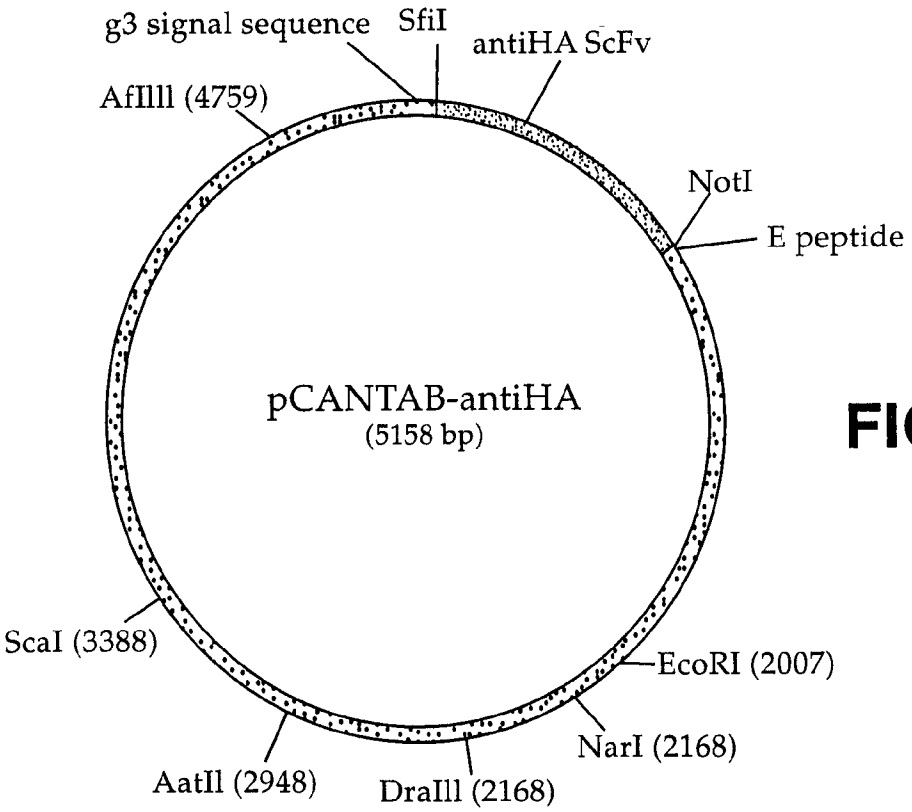

FIG. 17A

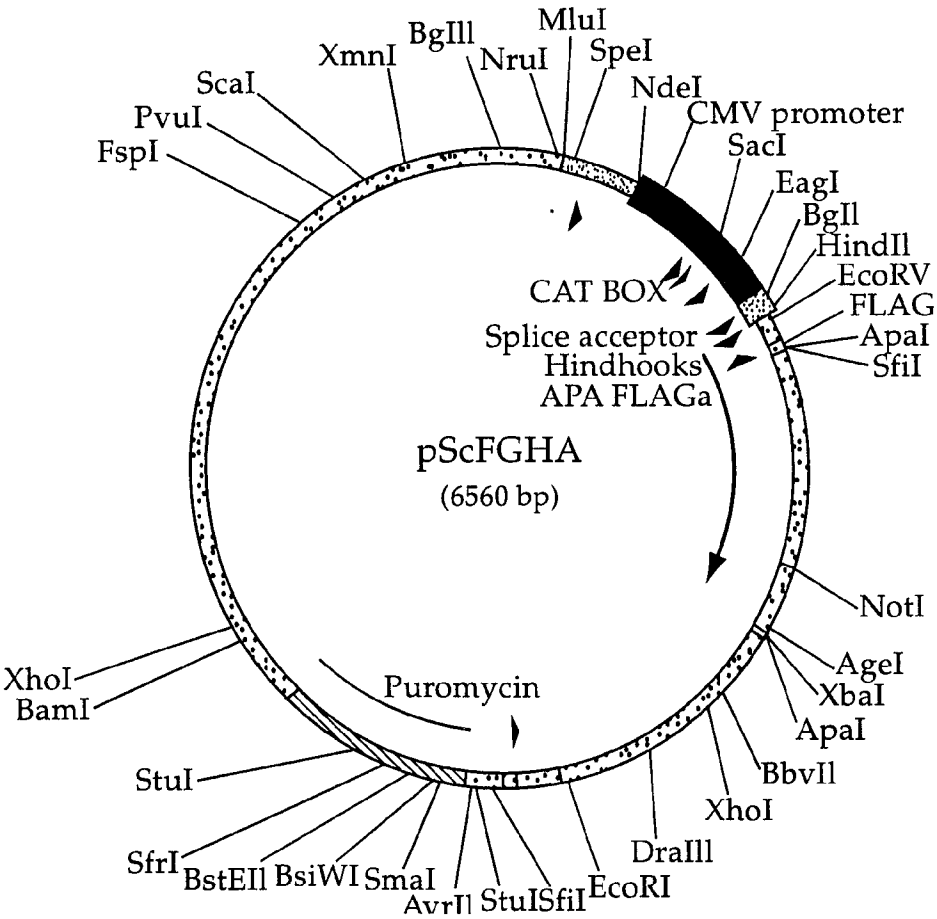

FIG. 17B

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/US 98/11024

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/86 C12N15/62 C07K14/075 C12N5/10 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 26281 A (GENVEC INC ;CORNELL RES FOUNDATION INC (US)) 29 August 1996	1-10, 13-29, 31-33
Y	see the whole document ---	11
Y	GILMORE R. ET AL.: "Co-translational trimerization of the reovirus cell attachment protein." THE EMBO JOURNAL, vol. 15, no. 11, 30 October 1996, pages 2651-2658, XP002078894 see discussion ---	11

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 September 1998	13/10/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mandl, B

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DOUGLAS J. T. ET AL.: "TARGETED GENE DELIVERY BY TROPISM-MODIFIED ADENOVIRAL VECTORS" BIO/TECHNOLOGY, vol. 14, November 1996, pages 1574-1578, XP002030944	29,31-33
Y	see the whole document, especially Fig. 1	30
Y	BERGELSON J. M. ET AL.: "Isolation of a common receptor for Coxsackie B viruses and Adenoviruses 2 and 5." SCIENCE, vol. 275, February 1997, pages 1320-1323, XP002078895 see the whole document	30
X	STEVENSON S. C. ET AL.: "Human adenovirus serotypes 3 and 5 bind to two different cellular receptors via the fiber head domain." JOURNAL OF VIROLOGY, vol. 69, no. 5, May 1995, pages 2850-2857, XP000608381 see the whole document	1-9, 13-23
X	WO 94 10323 A (IMP CANCER RES TECH ;SPOONER ROBERT ANTHONY (GB); EPENETOS AGAMEMN) 11 May 1994 see page 26, line 14 - page 27, line 22; figure 1; examples 1-4	1-9, 13-23, 26-28
X	MICHAEL S. I. ET AL.: "ADDITION OF A SHORT PEPTIDE LIGAND TO THE ADENOVIRUS FIBER PROTEIN" GENE THERAPY, vol. 2, no. 9, November 1995, pages 660-668, XP000607625 see the whole document	1,2,9, 13,14, 16-19, 21,26-28
A	WO 96 07734 A (GENVEC INC) 14 March 1996	1-28
A	WO 88 04692 A (IMMUNEX CORP) 30 June 1988 see the whole document	1-33
A	KLEIBOEKER S. B. : "Sequence analysis of the fiber genomic region of a porcine adenovirus predicts a novel fiber protein" VIRUS RESEARCH, vol. 39, 1995, pages 299-309, XP002079117 see the whole document	1-33

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 98/11024

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	BAI M. ET AL.: "Vitronectin receptor antibodies inhibit infection of HeLa and A549 cells by adenovirus type 12 but not by adenovirus type 2." JOURNAL OF VIROLOGY, vol. 68, no. 9, 1994, pages 5925-5932, XP002078897 ---	1-33
P,X	WO 98 07865 A (GENVEC INC) 26 February 1998 see the whole document, especially page 32, lines 24-37 ---	1-9, 13-23, 26-28
P,X	WICKHAM T. J. ET AL.: "Increased in vitro and in vivo gene transfer by adenovirus vectors containing chimeric fiber proteins." JOURNAL OF VIROLOGY, vol. 71, no. 11, November 1997, pages 8221-8229, XP002078898 see the whole document -----	1-9, 13-25, 29,31-33

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/ 11024

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 27 and 28, as far as an in vivo application is concerned, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-28

   A modified adenoviral fiber trimer; a composition comprising said trimer; an adenovirus comprising said trimer; a cell line exprissing a non-natural surface receptor; a method of propagating said adenovirus using said cell line; a method of purifying said adenovirus; and a method of inactivating said adenovirus.

2. Claims: 24, 25

   A cell line expressing a non-natural cell-surface receptor to which an adenovirus having a ligand for said receptor binds; and a method of propagating an adenovirus in said cell line.

3. Claims: 26-28

   A method of purifying an adenovirus having a ligand for a substrate wherein said substrate is used; a method of inactivating said adenovirus wherein said substrate absorbs said adenovirus.

4. Claims: 29-33

   A chimeric blocking protein comprising a substrate for an adenovirus fiber; and a method of interfering with adenoviral targeting using said protein.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte onal Application No
PCT/US 98/11024

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9626281 A	29-08-1996	US 5770442 A	23-06-1998
		AU 4980496 A	11-09-1996
		CA 2213343 A	29-08-1996
		EP 0811069 A	10-12-1997
WO 9410323 A	11-05-1994	EP 0672158 A	20-09-1995
		GB 2286593 A,B	23-08-1995
		JP 8506239 T	09-07-1996
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